Differentiating normal and abnormal ageing using a combined functional connectivity and neuropsychological biomarker

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Thesis submitted to the University of Sheffield for the degree of Doctor of Clinical Psychology

July 2013

Declaration

This work has not been submitted to any other institution

or for any other qualification.

Thesis Abstract

The impetus of this thesis is to ascertain the extent to which resting state neuroimaging reveals neuropsychologically relevant and clinically salient biomarkers to distinguish normal and abnormal ageing.

Section 1. A literature review was undertaken to characterise the neuropsychological correlates of resting state networks in persons with Mild Cognitive Impairment (MCI). The results suggest episodic memory performance decreases as a function of decreased coherence of the spontaneous correlations between medial parietal, temporal and frontal regions of the default mode network and that the posterior cingulate cortex (PCC) may be the most salient biomarker for cognitive decline due to Alzheimer's pathology. The conclusions suggest further work is needed to characterise the relationship between resting state imaging markers and semantic memory and to understand how semantic and episodic memory are mediated by resting state network connectivity in normal ageing.

Section 2. Based on the conclusions of the literature review an empirical study was devised to ascertain if clinical and age related variance in semantic and episodic memory is associated with disrupted connectivity of the PCC. The results demonstrated that whilst semantic memory is sensitive and specific to abnormal ageing, episodic memory impairment is more sensitive and specific to PCC connectivity in normal ageing and disease. The correlates of PCC connectivity and episodic memory in MCI were associated with disrupted connectivity with posterior memory structures, whereas in healthy ageing the connections between these regions were preserved. In those ageing normally, the inverse association with PCC connectivity with the caudate and insula may suggest cognitive efficiency, the association with frontal regions is in line with the frontal theories of ageing. These findings suggest the episodic memory correlates of PCC connectivity could indicate a clinically relevant biomarker for normal and abnormal ageing.

Acknowledgements

Firstly I must thank my supervisor Annalena Venneri, without whom this thesis would not have been possible. You have supported me to combine my great passions of neuropsychology and neuroscience in clinically meaningful ways. It has been a privilege to have an insight into the interface between brain and behaviour and I am truly grateful for the opportunity to consolidate former skills and learn entirely new techniques. Your vision for the role of clinical psychologists in neuropsychological assessment, formulation and intervention will continue to influence me for the rest of my career. I hope our collaboration and friendship will continue to grow.

I would also like to thank Claire Isaac for providing a vital link between this work and the Clinical Psychology Unit, your relaxed acceptance and down to earth approach has been reassuring and helpful.

I am especially grateful to Matteo DeMarco for transferring all the data from Venice, for training me to preprocess resting state images and how to define regions of interest. You were kind and patient and never complained about showing me the same thing twice. You made something complex and scary approachable and possible.

An enormously big thank you must go to my fabulous friend Katija. I must have made a permanent dent in your airbed! I don't know how you put up with me so often. You saved me so many vital hours in commuting so that I could stay late and start early on my data at the Hallamshire hospital.

Conducting an extensive piece of research whilst undertaking clinical work on placements also requires much negotiation and support from clinical supervisors, I would therefore like to thank my two placement supervisors Arleta Starza Smith and Yvonne Melia for being so sensitive and understanding about the demands of the research process.

I cannot overstate how much I owe my mum, sister and partner for their constant support and grounding throughout the thesis and the whole of training. I especially want to thank Jonathan for postponing many a weekend while I fretted at the laptop, for coping with post commute zombie partner most evenings and absorbing many domestic duties as the thesis drew nigh. I can't conclude without thanking my most loyal laptop companion – my dog Lincoln.

Structure and Word Counts

1. **Literature Review:** *Do functional connectivity studies of the resting state in mild cognitive impairment reveal neuropsychologically meaningful biomarkers? A systematic review of the literature.*

   Excluding references: 7,999

   Including references: 10,459

2. **Research Report:** *The neuropsychological correlates of posterior cingulate resting state connectivity in normal and abnormal ageing*

   Excluding references: 11,821

   Including references: 15,597

Total word count:

Excluding references and appendices: 19,820

Including references and appendices: 29,720

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Section 1

Literature Review

Do functional connectivity studies of the default mode network in mild cognitive impairment reveal neuropsychologically meaningful biomarkers? A systematic review of the literature.

Table of Abbreviations

AD = Alzheimer's disease, MCI = Mild Cognitive Impairment, MRI = Magnetic Resonance Imaging, fMRI = Functional Magnetic Resonance Imaging, RSN = Resting State Network, DMN = Default Mode Network, FC = Functional Connectivity, HC = Healthy Controls, CC = Cognitive Complaints, ICA = Independent Component Analysis, ReHo = Regional Homogeneity, GM = Grey Matter, ROI = Region of Interest, AVLT = Auditory Verbal Learning Test, CVLT = California Verbal Learning Test, DMPFC = Dorsomedial Prefrontal Cortex, DLPFC = Dorsolateral Prefrontal Cortex, aTP = anterior Temporal Pole, PFC = Prefrontal Cortex, PCC = Posterior Cingulate Cortex, PCu = Precuneus, FG = Fusiform Gyrus, LSN = Large Scale Networks, ALFF = Amplitude of Low Frequency Fluctuation.

Does resting state variance in mild cognitive impairment reveal neuropsychologically meaningful biomarkers? A systematic review of the literature.

Purpose: To characterise the neuropsychological correlates of resting state networks in persons with Mild Cognitive Impairment (MCI) through a systematically informed review of the empirical literature.

Method: A systematic strategy comprised of four classifications of terms, Neuropsychology, Mild Cognitive Impairment, Resting State Networks and Functional Magnetic Resonance Imaging, was devised to identify all relevant papers. Systematic search of Web of Knowledge database revealed 17 eligible studies.

Results: Findings suggest the episodic memory impairment in persons with MCI is linearly dependent on the integrity of connections between medial parietal, temporal and frontal regions of the default mode network (DMN). Episodic memory performance appears to decrease as a function of decreased coherence of the spontaneous correlations between these regions.

Conclusion: Despite methodological limitations, these exploratory findings offer insight into neural mechanisms which subserve the episodic memory impairment that characterises MCI. As the DMN devolves in a clinically relevant manner it may reflect an important marker of network pathology and organic cognitive decline.

Practitioner Points:

- May help clinical psychologists devise neuropsychological assessments to be more specific to the detection of functional markers of early neuropathological change.
- Assessments designed to assess DMN function may improve the ability to separate organic memory dysfunction such as those caused by Alzheimer's disease from functional episodic memory impairments that may arise from depression or anxiety.

- In order to improve early detection, the relationship between resting state imaging markers and semantic memory needs to be characterised.
- Further longitudinal studies with larger samples are required to substantiate these findings.
- Future studies would benefit from more statistically meaningful methods.

Alzheimer's disease (AD) is a neurodegenerative brain disease, in which progressive damage to the cerebral cortex results in the gradual loss of cognitive functions (Lopez & Becker, 2004). AD is the most common cause of dementia (Cummings, 2003), as life expectancy increases, the number of individuals at risk of developing AD is escalating (Nestor, Sheltons & Hodges, 2004). There is no curative treatment for this increasingly important public health issue (Davatzikos, Fan, Wu, Shen & Resnick, 2008). Although pharmacological and cognitive treatments may prolong onset, as the effects of AD pathology cause irreversible cerebral damage, such treatments need to be initiated as early as possible.

The onset of AD is commonly preceded by an interim phase identified as mild cognitive impairment (MCI; Petersen et al., 2001). MCI describes the onset and evolution of cognitive impairments which exceed those expected of an individual in consideration of their age and education despite being insufficient to interfere with activities of daily living (Peterson et al., 1999). MCI can be benign but frequently marks a transition between normal aging and dementia (Ganguli et al., 2004; Grundman et al., 2004; Geslani et al., 2005). Compared with cognitively normal persons, individuals with MCI have been found to have increased risk of developing AD (Petersen et al., 1999) and conversion rates of 6 – 25% per annum have been reported (Landau et al., 2010; Petersen 2009).

Research to improve the early detection of prodromal AD has focused on cognitive and neuroimaging biomarkers. However, in order to identify a prodromal phase of AD, a biomarker must be sensitive and specific to the earliest biological and/or neuropsychological changes associated with AD (Nestor et al., 2004). Whilst the quintessential imaging hallmark of AD is

atrophy in medial temporal and parietal lobes, in order for disease modifying treatments to work, the preclinical indices of disease need to be recognised before damaged neuronal cells manifest on magnetic resonance imaging scans (MRI) as atrophy (Binnewijzend, et al., 2012).

Functional markers may be detectable long before structural damage would be evident on MRI (Binnewijzend et al., 2012) and compared with task-based activation studies, task-free resting state imaging has been found to be a superior modality through which to detect prodromal pathological changes in the brain. Resting state imaging can be used to identify subtle functional abnormalities in the neural circuits that support the neuropsychological functions associated with MCI and AD (Agosta, Pievani, Geroldi, Copetti, Frisoni & Filippi, 2012).

Numerous studies have applied resting state methods to understand the neural integrity of brain networks in patients with AD and AD pathology has been found to progress preferentially along certain neural networks (Greicus et al., 2004). Recently, resting state imaging methods have been applied to the study of MCI patients (Wang et al., 2012) results suggest alterations in key resting state brain networks in persons with MCI may reflect the presence of AD pathology. Thus alterations in resting state networks in persons with MCI could reflect clinically meaningful biomarkers of incipient or prodromal AD.

The regions which demonstrate disrupted functional connectivity in those with MCI overlap considerably with regions which subserve the cognitive functions that are found to be most vulnerable to deterioration in the dementia prodrome. Most notably disconnections of the of the default mode network (DMN) are thought to subserve the early memory impairment in MCI and reflect the anatomical and cognitive processes involved in conversion to AD (Grecious et al., 2004). The DMN is comprised of parietal cortex including the precuneus and posterior cingulate, anterior cingulate, medial prefrontal cortex, hippocampus and thalamus. This network is associated with decreased activity in response to cognitive tasks which demand externalised attention (Grecious et al., 2004). The network is associated with internal processes such as episodic and autobiographical

memory, introspective cognition, past and future thinking, stream of consciousness, self referential processes, free association, monitoring internal and external environments, mind wandering and considering the perspectives of others (Grecious et al., 2004).

Most notably, the brain structures which are especially vulnerable to AD pathology and those which support the cognitive functions that characterise clinical symptoms of the disease, overlap with DMN structures (Buckner et al., 2008). Although variance in the DMN may represent the most salient biomarker for the cognitive sequelae of MCI and AD, relationships between DMN and non DMN structures and other distinct resting state networks may also reveal clinically meaningful relationships (Agosta et al., 2012).

However, in order to be clinically relevant, the extent to which alterations in these networks are associated with early and mild signs of cognitive impairment must be reviewed. Whether these theoretical brain behaviour relationships are reflected in actual relationships between resting state networks and cognitive performance of patients with MCI has only very recently been explored. Whilst descriptive narrative reviews have outlined key resting state predilection sites in AD and more recently MCI (Grecious et al., 2004; Liu et al., 2008; Damasioux, 2012) none of these have been systematic. Furthermore no review has examined the relationship between alteration in resting state networks and impaired cognition in persons with MCI. Therefore the purpose of the current review is to clarify whether the relationship between resting state functional connectivity in MCI is associated with the cognitive impairments which are neuroanatomically and behaviourally associated with this diagnosis. Understanding the relationship between connectivity in Resting State Networks (RSNs) and cognition in a prodromal phase of disease has important clinical implications for psychological and pharmacological assessment, early detection, intervention and outcome measures and will contribute to a theoretical understanding of the cognitive sequelae of prodromal neuropathology.

Rationale and aims of review

The aim of the current review is to characterise the relationship between alterations in resting state networks and cognitive performance in persons with MCI. The main hypothesis is that the hallmark MCI symptom of episodic memory impairment will be associated with disruption between key memory structures in the DMN, especially between medial parietal and medial temporal lobes, whether episodic memory is associated with connectivity of non DMN regions will also be assessed. In line with the characterisation of the DMN as task deactiviating and its role in internal mentalisation processes is it is unlikely that cognitive domains which are not predominantly memory based will correlate with its network of connectivity. In addition, as most studies sought samples of amnestic rather than multi-domain MCI it is unlikely that variance in other neuropsychological domains will vary with the connectivity of other networks.

Method

A systematic search strategy was devised to access all papers which may contribute to the question; *does resting state variance in mild cognitive impairment reveal neuropsychologically meaningful biomarkers?*

Search Strategy

The searches for this review were undertaken in April 2013. As this is a relatively new area of investigation and no known study of resting state fMRI has been published prior to the year 2000, the search time frame encompassed papers from the year 2000 to the present up to and including those published in March 2013. As Web of Knowledge includes the Science Citation Index, the Social Sciences Citation Index and MEDLINE it was considered to cover sufficient breadth to be the sole search engine for the literature search. Subsequent coverage checks through PubMed (n=261) and PsycINFO (n=71) only revealed duplicates. The following search terms, combined with Boolean operators were entered into the database, (mild cognitive impairment* OR MCI* OR

age associated cognitive decline OR dementia prodrome OR incipient dementia) AND (resting state OR resting state network* OR task-free network* OR default mode network* OR default mode connectivity OR functional connectivity OR deactivation) AND (fMRI* OR functional magnetic resonance imaging* OR MRI) AND (cognitive* OR neuropsychology OR neuropsychological OR behavioural).

Inclusion and exclusion criteria

The search returned 419 papers. After reviewing titles and, when necessary abstracts, 39 studies required full text assessment. Functional magnetic imaging studies (fMRI), employing resting state paradigms which directly assessed relationships between variance in resting state networks and specific measures of cognition in persons with MCI were retained. These criteria yielded 17 papers, a PRISMA diagram (Moher, Liberati, Tetzlaff & Altmann, 2009) of the filtering process can be found in Figure 1.

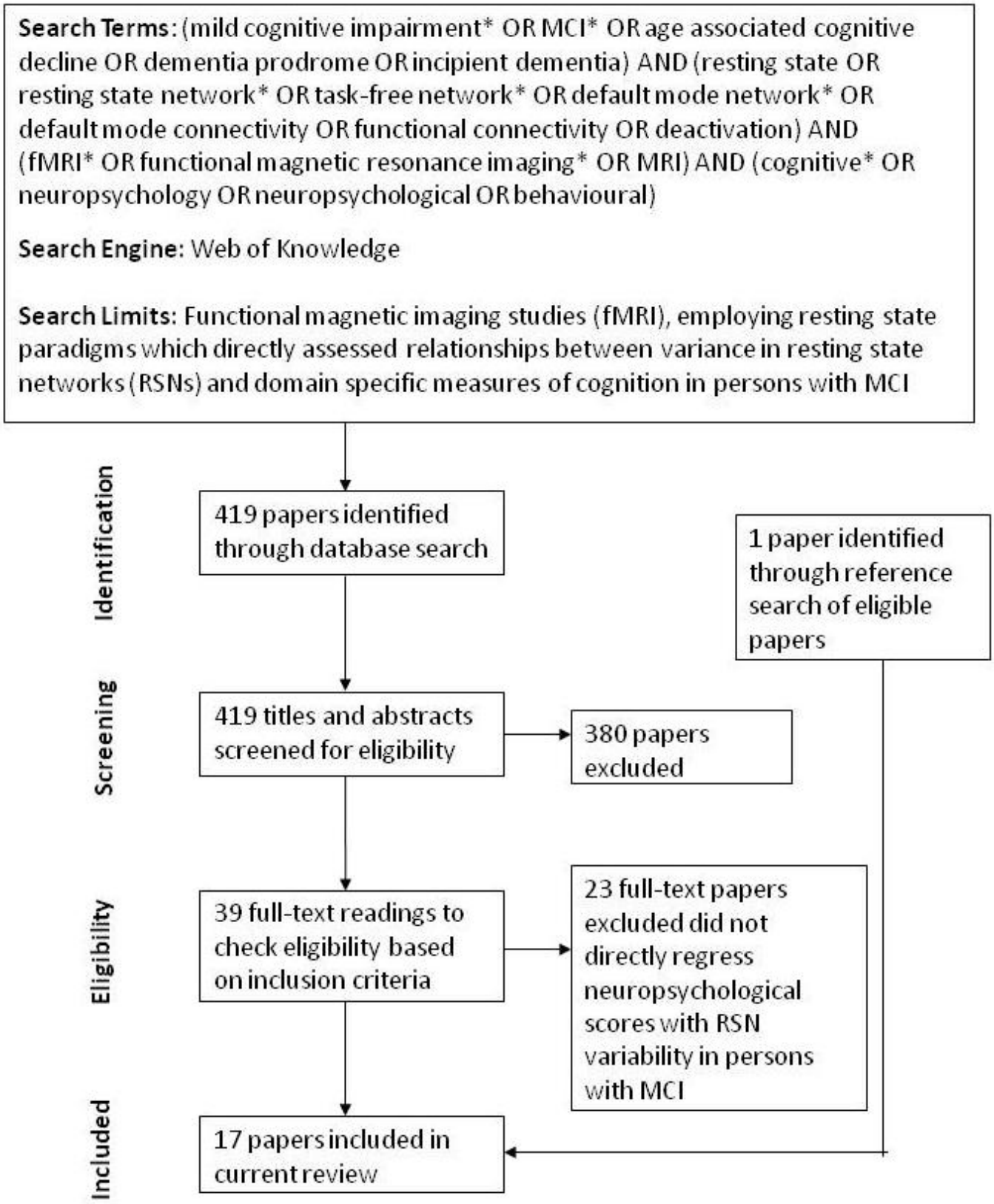

Figure 1. PRISMA diagram (Moher et al., 1998) detailing the review filtering process

Quality control

The majority of studies were cross sectional, all used correlational analysis to identify relationships between variance in RSNs and cognitive performance either within a group of persons with MCI or across diagnostic groups of MCI, healthy controls (HC) and/or AD. Several studies were longitudinal although, typically behavioural correlations remained cross sectional as associations between RSN and cognition were only followed up in two studies (Bai et al., 2011; 2011a). As variability in quality was evident, Downs and Black's quality control checklist (1998) was adapted to quantify the key methodological issues inherent in these studies (Appendix 1). A second rater was asked to apply this checklist to half of the review papers. This individual was a Post Doctorial Research Fellow and Honorary Clinical Psychologist at the Royal Hallamshire Hospital in Sheffield. This individual has a PhD in neuropsychology and their clinical and research activities are in the field of ageing, dementia and neuropsychology. Papers were assigned a number based on their alphabetical appearance in the reference list, the RANDBETWEEN function in Excel was used to randomly select 9 papers for second rating. Pearson correlation revealed inter-rater reliability of quality scores was high ($r=0.98$, $p<0.001$). A Data Extraction Table summarising, critiquing and rating all pertinent study details can be found in Table 1. Whilst variability in quality was evident (M=20.59, SD=3.57, R=29-14), this bore no relevance to the consistency of findings across studies. Consequently, no study was excluded on the basis of quality scores, instead the relationship between methodological issues and the robustness of findings will be considered.

Results

The findings of the current review are presented in line with the neuropsychological domains which were found to be associated with variance in RSNs namely, Episodic Memory Retrieval in the DMN and Beyond, Episodic Memory Encoding, Executive Function and Other Associated Cognitive Domains. As several important methodological issues and limitations are

intrinsic to all studies, the implications of these on the findings will be critically appraised after the results section.

Authors	Sample	MCI Criteria	Resting State Imaging Analysis	Cognitive Measures	Connectivity & Cognitive Correlations Across Groups	Connectivity & Cognitive Correlations Separate Groups	Control for GM in behavioural correlations?	Correct for MC RSN - NP	Critique	Quality score (0-34)
Binnewijzend et al., (2012)	MCI(23) HC(43) AD(39)	Petersen et al. (2001)	ICA then dual regression	Digit Span (DS: F & B) AVLT (I* & D*) VAT* Picture naming CF* TMT (A & B) Stroop Rey Figure (C)	Across AD,MCI,HC +ve r Lower FC in DMN lower performance on DS B, Stroop, TMT A&B, VAT, AVLT I & D CF & Rey Figure C	No r MCI No r HC +ve r AD FC & Rey copy	Yes WB	Yes - No	Pros: Longitudinal follow-up identify stable & converting MCI Cons: Small MCI sample Sample heterogeneity Dual regression assumes commonality of maps	29
Xie et al., (2012)	MCI(43) HC(33)	Petersen, et al. (2004)	ROI Insula	RAVLT (D*) Rey Figure (I*) TMT (A*&B*) DS* DSMT* Created index scores For EM, EF, PS, WM from these tests	N/A	MCI +ve r insula coupling with L DMPFC, L DLPFC, L aTP, B a PFC and EM index score No r between insula couplings and HC	Yes of the right posteria insula subregions	No - No	Pros: looked for ROI GM differences and controlled for these in regression Comparatively large MCI sample Index scores reduce random variability Computed separate regression in HC – so disease inferences can be made Cons: EM index score may be too non specific, verbal & visual I & D Did not assess WB GMV	25
Bai et al., (2011a)	MCI(26) HC(18)	Petersen (1999)	Hippocampal ROIs	AVLT (D)* Rey Figure (C & D*) TMT A*&B* SDMT* CDT DS	N/A	MCI +ve r lower HiP –PCC connectivity Lower AVLT-D No r in HC	Yes for HiP	Yes - No	Pros: Control HiP GMV Longitudinal design Described and removed converters from baseline Undertook analyses for HC so disease inferences can be made Cons: Did not explain sample overlap with Bai et al., 2011	24
Liang, Wang, Yang, Jia & Li (2011)	MCI(14) HC(14)	Petersen et al. (2004)	ROI B DLPFC	CVLT (I*, SD*, LD*) CDT*	N/A	After control for GM +ve r MCI lower connectivity of right DLPFC R IPL & CVLT I & SD	Yes	Yes - Yes	Pros: Controlled GM at behavioural level Looked for right and left DLPFC differences Covered episodic encoding and recall Cons: Small sample	23

Authors	Sample	MCI Criteria	Resting State Imaging Analysis	Cognitive Measures	Connectivity & Cognitive Correlations Across Groups	Connectivity & Cognitive Correlations Separate Groups	Control for GM in behavioural correlations?	Correct for MC RSN - NP	Critique	Quality score (0-34)
Agosta et al., (2012)	MCI(12) HC(13) AD(13)	Petersen et al(2001) + 1 abnormal AD biomarker	ICA then ROI based on known RSN functional associations	Babcock Story Recall (I & D) Rey's word list (I & D) Rey figure (C & D) Trail Making (TMT) A & B Phonological fluency CF Token Test Combined select tests for index score approach Memory Z score*	None found	N/A	Yes WB	Yes - Yes	Pros: Index scores reduce floor/ceiling effects Biomarker improves certainty of AD pathology Included semantic measure Cons: Small sample No standardised protocol for limiting ICAs	22
Bai et al., (2011)	MCI(26) HC(18)	Petersen (1999)	ICA to identify DMN	AVLT (D)* Rey Figure (C & D*) TMT A*&B* SDMT* CDT DS	N/A	Baseline to Follow up MCI +ve r lower PCC PCu FC & mean DMN lower AVLT D scores	No	Yes - No	Pros: Longitudinal design Described and removed converters from baseline Cons: Possible sample heterogeneity 26 stable MCI for 20 months Did not covary for GM in behavioural correlations No HC regressions	22
Bai et al., (2009)	MCI(30) HC(26)	Petersen (1999)	ROI PCC	AVLT (D*) Rey Figure DS SDMT* TMT (A & B*) CDT	N/A	+ve MCI lower FC between PCC L MTG and poorer performance In TMT B and SDMT No r HC	No	Yes - No	Pros: Comparatively larger clinical sample Looked for associations in HC group Hypothesis driven ROI Cons: did not control for GM or MC Small range of episodic measures	21
Liang, Wang, Yang & Li, (2012)	MCI(16) HC(16)	Petersen et al., (2004)	ROI of B IPS, B AG, B SG	CVLT (I*, SD*, LD*) CDT*	N/A	-ve r MCI higher connectivity differences AG & rPCu with assoc lower scores CVLT I & SD	No	Yes - Yes	Pros: Looked beyond DMN but still hypothesis driven Covered episodic encoding and recall Cons: Small sample Not controlled for GM atrophy in behavioural FC correlations No analysis of FC cognition in HC	21

Authors	Sample	MCI Criteria	Resting State Imaging Analysis	Cognitive Measures	Connectivity & Cognitive Correlations Across Groups	Connectivity & Cognitive Correlations Separate Groups	Control for GM in behavioural correlations?	Correct for MC RSN - NP	Critique	Quality score (0-34)
Blautzik et al., (2013)	MCI(13) HC(12)	Petersen 2001	ICA	Verbal Fluency Boston Naming Word List (Learning*, recall* & Recognition*) Constructional Praxis & recall* CDT*	N/A	None survived Bonferroni correction	No	Yes - Yes	Pros: Corrected for MCs Longitudinal design for RSN reliability Cons: Very small sample Did not control for GMV ICAs could be under or over inclusive	20
Wang et al., (2012)	MCI(14) HC(14)	Petersen 1999	ROI PCC	CVLT (I*, SD*, D*) CDT**	N/A	+ve MCI lower FC L STG-PCC & D ACC-PCC & I	Yes	Yes - No	Pros: Longitudinal design Controlled for GM volume Cons: Did not compute brain behaviour correlations at follow up No MC control Only used MMSE for cognitive variable	20
Wang et al., (2012a)	MC(30) HC(47)	Used their own standardised battery and clinician interview	Frequency-dependent neural networks, wavelet based correlations	CDT* AVLT (I*, D*, R*)	N/A	+ve MCI whole brain topological connectome and nodal strengths of AG, MTG, ITG MFG with AVLT I & R -ve fitted network path length & AVLT R	No	No - No	Pros: Unbiased whole brain analysis Controlled cardiac and respiratory signal Comparably large sample Discusses consistency of findings across methods Cons: MMSE criteria for HC is unusually broad range 20-30 low scores may suggest underlying pathology Did not correct for MC at nodal or behavioural level	20
Yao et al., (2013)	MCI(27) AD(35) HC(27)	Petersen et al., 1999	ROI Amygdala	AVLT (I & D*)	MCI + AD +ve r Amygdala FC & L STG, PCG & AVLT-D	Computed for separate AD and MCI not significant	No	Yes - No	Pros: Novel but meaningful ROI Sound theoretical rationale Cons: Did not included HC in regression or compute separate HC analysis Did not account for Amygdala subregions Did not control for Amygdala or WB atrophy	20

Authors	Sample	MCI Criteria	Resting State Imaging Analysis	Cognitive Measures	Connectivity & Cognitive Correlations Across Groups	Connectivity & Cognitive Correlations Separate Groups	Control for GM in behavioural correlations?	Correct for MC RSN - NP	Critique	Quality score (0-34)
Wang et al., (2013)	MCI(18) HC(16) CC(23)	In line with published criteria	ICA to identify DMN	CVLTtot* CVLT (SD*& LD*)	Across MCI,CC,HC +ve r CVLT-tot/SD&LD DMN FC R HiC, R HiG & R Th	N/A	N/A no differences found	Yes - No	Pros: Tested for GM differences Unbiased approach Inclusion of possible pre MCI group Covaried for numerical non statistical demographic differences Cons: No control for MC More information about MCI criteria needed Limited cognitive measures	18
Zhang et al., (2012)	MCI(19) AD(23) NC(21)	Petersen et al.,(1999)	ReHo WB	AVLT (I, D*, Rec)	MCI & AD +ve r lower ReHo in B PCC/PCu L IPL & AVLT-I & D	-ve r MCI ReHo PCC/PCu & AVLT-I	No	No - No	Pros: Original index of connectivity Considered consistency with other methods Considered r with GM and ReHo Cons: Did not include HC in regression or compute separate HC analysis Did not control for GM Did not correct for MC	18
Yan, Zhang, Chen, Wang & Liu, (2013)	MCI(18) HC(18)	In line with Petersen et al.	ICA and causality analysis	AVLT (I, D, Rec)	N/A	+ve r causal influence strength HiP to PCC/PCu and AVLT-I MTG to FG with AVLT-D	No	Yes - No	Pros: Demonstrated causal directional relationships Cons: Did not control for GM or MC, limited behavioural measures	17
Jin et al., (2012)	MCI(10) HC(8)	Petersen 2001	ICA from slices perpendicular to long axis of hippocampus	Boston Naming CVLT (D & T)* Logical Memory I & II Controlled Oral Word Association Test Animal naming TMT A & B SDMT Block Design Benton Visual Recognition	Across MCI & HC +ve r lower FC between DMN and L PFC, L MTG and R AG with lower CVLT D & T	N/A	Yes MTL	Yes - No	Pros: Acquired slices to optimise detection of HiP Broad selection of cognitive measures Cons: Very small sample size only corrected for MTL GM did not correct for MC for FC cognition correlations May overestimate ICs	16

Authors	Sample	MCI Criteria	Resting State Imaging Analysis	Cognitive Measures	Connectivity & Cognitive Correlations Across Groups	Connectivity & Cognitive Correlations Separate Groups	Control for GM in behavioural correlations?	Correct for MC RSN - NP	Critique	Quality score (0-34)
Chen et al., (2011)	AD(20) MCI(20) HC(20)	Petersen 1999	Automatic parcellation 116 ROIs then Pairwise ROIs	MMSE* RAVLT D*	Combined AD,MCI,HC + r MCI & HC increased & decreased connectivity index & RAVLT D	N/A	No	No - No	Pros: Independent of DMN or MTL hypothesis Overall group discrimination good, moderate sample size Cons: Only one memory measure No control for GM or MCs	14

Abbreviations: GM=Grey Matter, MC=Multiple Comparisons, RSN=Resting-state network, NP=Neuropsychology, ICA=Independent Component Analysis, ReHo=Regional Homogeneity, F=forward, B=Back, I=immediate, D=Delay, SD=Short Delay, LD=Long Delay, AVLT=Auditory Verbal Learning Test, VAT=Visual Association Test, CF=Category Fluency, TMT=Trail Making Test, C=Copy, +ve=positive, -ve=negative, r=Correlation, FC=Functional Connectivity, DMN=Default Mode Network, WB=Whole Brain, GMV=Grey Matter Volume, GM=Grey Matter, ROI=Region of Interest, CDT=Clock Drawing Test, DSMT= Digit Symbol Modalities Test, EM=Episodic Memory, EF=Executive Function, PS=Processing Speed, WM=Working Memory, L=Left, R=Right, B=Bilateral, DMPFC=Dorsomedial Prefrontal Cortex, DLPFC=Dorsolateral Prefrontal Cortex, aTP=anterior Temporal Pole, PFC=Prefrontal Cortex, PCC=Posterior Cingulate Cortex, Hip=Hippocampus, HiG=Hippocampal Gyrus, PCu=Precuneus, MTG/L=Medial Temporal Gyrus/Lobe, IPS=Inferior Parietal Sulcus, AG=Angular Gyrus, SG=Supramarginal Gyrus, ACC=Anterior Cingulate Gyrus, Th=Thalamus, STG=Superior Temporal Gyrus, ALFF=Amplitude of Low Frequency Fluctuation.

Table1. Data Extraction

Episodic Memory

Episodic memory dysfunction is the characteristic and often earliest indication of incipient AD. All studies under review computed correlations between DMN and neuropsychological measures of episodic memory. Each study used well established neuropsychological tests of verbal episodic memory including California Verbal Learning Test (CVLT; Delis, Kramer, Kaplan & Ober, 1987; 2000; n=5), Auditory Verbal Learning Test (Rey, 1958; AVLT; n=10), Word List Learning (Morris et al., 1989; n=1) and Babcock Story Recall (Babcock & Levy, 1940; n=1). Rey-Osterrieth (1993) Complex Figure delay scores were also included in several batteries as a measure of visuospatial episodic memory retrieval. Whilst all protocols included measures of episodic retrieval (delayed scores) fewer included measures of episodic encoding (immediate scores n=9) and recognition (n=4).

Episodic retrieval. Using an unbiased Independent Component Analysis (ICA) approach, across AD, MCI and HC groups, linear dependence was evident between lower functional connectivity FC in the DMN and episodic memory as measured by AVLT-D (Binnewijzend et al., 2012). Whilst age and sex were covaried, there was no correction for multiple comparisons. However, as correlation strength was only slightly reduced after correction for GM volume, the results can only partially be attributed to structural density. Within individual groups, the only significant association between functional connectivity in the DMN was with the copy trial of Rey Complex Figure in the AD group. The longitudinal design revealed that MCI conversion rates were in line with published criteria which suggests a third of the MCI sample had AD pathology. This study attained a high quality rating although findings would be further strengthened with a longer follow-up period to trace differences between other stable and converting MCIs and through longitudinal study of brain behavioural correlations.

After application of Regional Homogeneity (ReHo), to map regional activity across the whole brain, of persons with AD, MCI and HC, indices of significant difference between the groups

were regressed against cognitive performance of the AD and MCI groups (Zhang et al., 2012). After controlling for age, sex and grey matter volume (GMV), significant results were found between the ReHO of the DMN and episodic memory for the MCI group. Lower ReHo between bilateral posterior cingulate cortex (PCC) and precuneus (PCu) and left inferior parietal lobule was related to lower AVLT delayed recall scores.

The PCC is an important correlate of episodic memory and is central to the DMN. Based on a priori hypotheses about such medial parietal dysfunctions a PCC region of interest (ROI) was found to be linearly dependent with cognition (Wang et al., 2012) and spatially distinct neural structures. Correlation analysis in the MCI group revealed lower episodic memory scores were associated with decreased PCC FC with the left superior temporal gyrus (Wang et al., 2012). However, whilst the authors compared differences in PCC connectivity with and without GM control, it is unclear if they used the GM controlled connectivity scores in their cognitive correlations so the extent to which these findings precede structural deterioration cannot be determined.

Further evidence about the role of PCC disconnectivity in episodic memory decline was found in the longitudinal study that assessed the differences in neuropsychological impairment and connectivity over time (Bai et al., 2011). In this study, ICA was used to identify the DMN at participant level, before comparing GM controlled differences in MCI and HC DMN at baseline and follow up. At baseline compared to controls, MCI patients had hyper connectivity between the DMN and the PCC and PCu, however after a 20 month follow up these regions reflected the most notable disruption in the form of significantly reduced DMN connectivity (Bai et al., 2011). In addition, across the baseline and follow-up period, decreasing DMN connectivity between the PCC and PCu was significantly positively associated with episodic memory impairments. Whilst HCs showed a small level of DMN change, the amplitude of DMN change in the PCC and its relationship with episodic memory impairment in MCI suggests abnormality may be rapidly

expressed through this network. The six individuals who converted from MCI to AD over the 20 month period were excluded from baseline data and were not follow up scanned. Significant sample heterogeneity is likely to be evident in the sample of 26 MCI persons who did not convert in this period.

It is apparent the same sample was also used in a separate study (Bai et al., 2011a). In this study, six hippocampal sub regions were used as seeds for ROI analysis. After control for demographic variables, longitudinal change in the sub-regional networks connected with these seeds and longitudinal change in neuropsychological scores revealed decreased FC between hippocampal subdivisions and the PCC were significantly associated with reduced episodic memory scores (Bai et al., 2011a). The lack of change in hippocampal sub-regional connectivity with the HCs suggests this reflects mechanisms of disease rather than ageing, although separate neurobehavioral correlation in this group would have further substantiated this evidence. Whilst sample overlap may confuse the generalisability of these results, that way in which two distinct analysis approaches revealed neurobehavioural correlation between PCC connectivity with regions of the DMN and episodic memory suggests the centrality of these relationships to the mediation of this memory domain.

When cognitive test scores were correlated with DMN FC across MCI and HC participants, Jin, Pelak & Cordes (2012) found significant associations between left lateral PFC, right angular gyrus and left middle temporal gyrus with delayed recall CVLT delayed and learning efficiency scores. The association with the angular gyrus and DMN structures was also evident in the significant and near significant relationship between the angular gyrus and right PCu with CVLT short and long delay recall ($p=0.058$) respectively. Grey matter and multiple comparisons were corrected in these analyses, and given the small sample ($n=16$), the corrected significance suggests a robustness of these relationships. However, this study attained the lowest quality rating and therefore caution must be exercised when interpreting the findings. The relationship between

decreased FC of the angular gyrus and right PCu with lower episodic memory also approached statistical significance in another study (Liang, Wang, Yang & Li, 2012). Further studies considering effect size and power are needed to ascertain if these are indeed meaningful brain behaviour relationships. Indeed, the fact that no study under review reported effect sizes limits the extent to which the findings can be interpreted as indicating reliable or robust relationships and as such, all future studies would benefit from the use of more stringent data reporting standards.

In a study which identified DMNs in a sample of MCI, HC and psychometrically normal individuals with subjective cognitive complaints (CC; Wang et al., 2013) increased disconnection in the DMN FC with the right hippocampus was expressed as a function of disease status with CC status being intermediary between MCI and healthy controls (Wang et al., 2013). Across all groups, memory performance measured by the CVLT-II was associated with DMN connectivity. Episodic memory scores for long and short delay and total scores were positively associated with higher DMN connectivity with the right hippocampus, right hippocampal gyrus and right thalamus (Wang et al., 2013). A subsequent right hippocampal ROI was identified from the difference between MCI and healthy controls and correlated with cognitive scores. Partial correlations revealed right hippocampal DMN connectivity was positively associated with episodic memory short and long delay and total scores. As there were no between group differences in GM atrophy in the DMN, these results suggest functional changes between brain network and cognition may precede structural atrophy. The findings also suggest pre MCI participants may represent an even earlier 'therapeutic opportunity' (Wang et al., 2013, p.759).

Episodic retrieval beyond the DMN. Xie et al. (2012) investigated the extent to which disrupted functional connectivity of the insula is associated with cognitive impairment in persons with MCI. For patients with MCI episodic memory impairment was significantly positively associated with decreased intrinsic connectivity of overlapping regions of the anterior and posterior insula networks with the bilateral anterior prefrontal cortex (PFC), left dorsomedial PFC (DMPFC),

left dorsolateral PFC (DLPFC) and left anterior temporal pole (aTP). The authors suggest their composite scores may have increased power through reduction of ceiling and floor issues and lowering random variability. However their episodic memory composite may be somewhat confounded by inclusion of a measure of immediate visual memory. As the study used insula ROIs only insula volume was covaried in analyses, although this does not rule out the effects atrophy in other regions may imply for connectivity. Additionally, no corrections were applied for the number of comparisons. As the lack of association between insula connectivity and HCs was reported, one can place more confidence in interpreting these findings as being relevant for pathology rather than ageing.

The relationship of the DLPFC in distributed networks supporting episodic memory was also implied by the results of a frontal ROI study (Liang, Wang, Yang, Jia & Li, 2011). After controlling for GM, the connectivity between a DLPFC ROI and right parietal dysfunction was associated with CVLT short delay. In the only study to look at the FC of an amygdala ROI, regression across AD and MCI groups revealed amygdala FC of superior temporal gyrus and precentral gyrus was associated and AVLT delayed recall scores (Yao et al., 2013). However, these associations were not evident in either group when separated.

Whilst many of the studies under review explored functional connectivity, one paper explored the causal direction of neural interactions. The strength of causal influence exerted from the middle temporal gyrus to the fusiform gyrus (FG) was positively associated with episodic memory (AVLT delayed scores; Yan et al., 2013). The application of multivariate Granger analysis allowed the direction of effective connectivity to be characterised, the findings would be strengthened by GMV control, increased power and computation/reporting of brain behaviour correlations for the HC group in order to make ageing/ disease inferences. Similarly, a trend for an association with decreased FG FC in the DMN and lower delayed recall scores approached significance in a very small MCI sample (Jin et al., 2012).

Large scale network (LSN) analyses, sensitive to change across the whole brain, were able to differentiate between AD, MCI and HC (Chen et al., 2011). Across these groups the more the connectivity index was decreased, the more severe the episodic memory impairment. Such unbiased whole brain analysis circumvents confirmation biases associated with certain hypotheses and does not impose limits on number or region of neural connectivity. However, whilst the relationship with decreased connectivity indices appears useful at the level of classification between diagnostic groups they may be too unspecific to contribute to an understanding of the mechanisms of normal and abnormal ageing. Whilst it could be inferred that such findings counter the specificity hypothesis of the DMN, such a lack of specificity would be contrary to neuropsychological and neurological knowledge and theory about the regional specialisation between brain and behaviour in adulthood. The relationship between episodic memory impairment and the connectivity index is thus likely to be a reflection of disconnection between the DMN or other neuropsychologically relevant networks. Further analyses are needed to control for the extent to which default mode network functional connectivity mediates the relationship between larger scale network signal and episodic memory.

Episodic encoding. Immediate memory scores on neuropsychological tests are devised to approximate episodic encoding. Many of the studies which found associations between RSNs and measures of episodic encoding have already been discussed in relation to episodic retrieval, to avoid repetition, critical considerations will not be reiterated. Of the 9 studies that regressed a measure of immediate memory against RSNs, seven found significant positive relationships.

Zhang et al. (2012) reported distinct significant results between DMN and immediate memory for their MCI and AD groups. For the MCI group, lower ReHo between medial PFC, bilateral PCC and PCu and left inferior parietal lobule was related to lower AVLT immediate scores. These findings are somewhat convergent with the ROI study of PCC connectivity which found immediate memory was associated with PCC FC in the left anterior cingulate and right inferior

parietal lobe (Wang et al., 2012). Further evidence for the centrality of PCC dysfunction in immediate memory was reflected in the positive association between strength of connectivity between the PCC/PCu and hippocampus with AVLT immediate scores (Yan et al., 2013). When regressed across AD, MCI and HC groups, whole network default mode associations were demonstrated between the mean regional functional connectivity in the DMN and AVLT-immediate scores (Binnewijzend et al., 2012)

In addition to the relationship of medial PFC connectivity, disruption of the DLPFC and the right inferior parietal lobule was significantly associated with the CVLT immediate recall (Liang et al., 2011), this relationship survived correction for GMV and multiple comparisons.

Several studies demonstrated that disrupted connectivity in the angular gyrus was relevant for impaired episodic memory encoding. Liang et al. (2012) found that after post-hoc correction for multiple comparisons, higher disconnection between the angular gyrus and the right PCu in the DMN was significantly associated with lower immediate recall. Similarly Wang et al., (2013a) found across the whole brain, the fitted nodal strength of the angular gyrus and inferior temporal gyrus were positively correlated with AVLT-immediate scores.

Executive Function

Whilst half the studies included measures of executive function, only two found associations with these scores and variance in RSNs. The connectivity between the left DLPFC and a cluster of voxels in the left thalamus was significantly correlated with the Clock Drawing Test (Liang et al., 2011) and the connectivity of the PCC and left middle temporal gyrus was found to be significantly positively associated with the symbol digit modalities test and inversely associated with the Trail making test-B (Bai, Watson, Yu, Shi, Yuan, Zhang, 2009). Although the relationship reported by Liang et al (2011) survived Bonferoni correction the frontal-striatal associations between DPFC and thalamus and CDT did not remain significant after GM control. However, the associations

demonstrated by Bai et al (2009) must be interpreted with caution as they were not corrected for multiple comparisons and they would be unlikely to survive this threshold.

Other Cognitive Domains

Further evidence for medial parietal involvement in the DMN across a continuum of AD pathology was described by Binnewijzend et al. (2012). Across AD, MCI and HC groups, linear dependence between lower FC in the DMN and tests of disease severity and cognitive function was evident across varied of neuropsychological domains, backward Digit Span, Stroop Word and Colour Word, Verbal Association Test, Category Fluency and the copy trial of Rey Complex Figure scores.

No Correlations

Two of the studies under review found no significant correlations between neuropsychological impairment in MCI and variance in resting connectivity. Both were of intermediate quality. Agosta et al. (2012) investigated the connectivity of the DMN in patients with AD, MCI and HCs and found, when compared with controls, the connectivity of the precuneus (PCu) in the DMN was significantly reduced in persons with MCI. However this reduction was not correlated with neuropsychological impairment. In the other study, supplemental material suggests there were several positive correlations between neuropsychological tests and functional connectivity in resting state networks however none survived Bonferroni correction for multiple comparisons (Blautzik et al., 2013). Sample heterogeneity, perhaps introduced by MCI multi domain impairments at baseline and very small sample size may suggest the non corrected associations reflect interesting exploratory data.

Methodological issues

The majority of studies were cross sectional and correlational. The lack of experimental

design means that causality cannot be inferred and tertium quid explanatory factors cannot be excluded. Unfortunately not all studies extended their cross sectional designs to exploration of brain behaviour relationships. For studies which only regressed neuropsychological scores with RSNs in persons with MCI, one cannot attribute these relationships to disease process as they could reflect normal ageing processes. Additionally, as no study compared HCs with a younger control group, it was not possible to ascertain how these relationships vary with normal ageing. Future studies would need to include a younger comparison group in order to differentiate changes as a function of ageing and disease.

Correlational designs also present the problem of multiple comparisons, the majority of studies used statistical means to correct for this. However, small sample sizes may have often led meaningful findings to be lost insufficient power. Therefore studies which did not control for this issue and significant trends have been reported with caution.

Whilst all studies used RS fMRI, several different methods of analysis were employed, each with their own advantages and limitations. The majority of studies employed an a priori region of interest (ROI) or un-biased hypothesis free independent component analysis (ICA) other studies combined approaches. Three studies analysed frequencies at the whole brain level through nodal strength, ReHO and Amplitude of Low Frequency Fluctuation (ALFF). For a more detailed review of RSN imaging methods see (Liu et al., 2008). Convergence of results with the contents of this review and other relevant studies suggest these methods produce comparable results. However, within-study comparison between different analysis techniques are needed in order to obtain confidence that results are solely attributable to disease status rather than artefacts of analysis methodologies.

Despite methodology, all brain behaviour correlations were computed by correlating the behavioural score with the z value of the connectivity between significantly temporally correlated brain regions between or across groups. The results would be more ecologically valid if they were

obtained from multiple regression analysis where behavioural scores can be understood in line with the variance of the full-scale information of the brain rather than statistically extracted Z scores.

The concept of MCI has been the source of much controversy. Whilst the term is now considered meaningful in clinical and research contexts (Petersen, 2004), sample heterogeneity continues to confound results as individuals with MCI may not have AD pathology. This issue was manifest across all studies and adherence to published standards of MCI diagnosis was universally respected, several also employed longitudinal design to document proportion of sample conversion (i.e. Petersen et al, 1999; 2001; Petersen, 2004).

As atrophy could affect the interpretation of results in functional imaging studies (He et al., 2007) several studies controlled for grey matter atrophy in their functional connectivity analyses at the level of their imaging data or as a covariate in the behavioural imaging regressions. Without control for atrophy it is not possible to ascertain whether variance between regions is independent from structural volume loss (Wang et al., 2012).

Perhaps the main limitation of resting state studies is that there is no reliable control for subconscious thought and uncontrolled cognitive processing (Liang et al., 2011). It is therefore not possible to exclude the interpretation that group differences may reflect between group differences in spontaneous thought rather than RSN differences (Bai et al., 2009). A further fundamental issue could be reflected in the assumption that relationships between neuropsychological scores and RSNs are linear. Whilst consistent evidence for linear dependence was found, perhaps RSNs also confer other subtle complex capacities than are not typically measured by neuropsychological tests.

Discussion

Despite limitations, patterns of findings converged across review studies that are consistent with

brain behaviour relationships reported in studies using different imaging modalities, analyses and neuropsychological measures in health and disease.

In line with the hypothesis, impaired episodic memory was frequently significantly associated with disruption of key DMN structures, most notably between the PCC and medial temporal and parietal structures. The PCC is a key node in the DMN (Grecious et al., 2004) and structural and functional imaging findings attest to the role of PCC in AD and MCI pathophysiology (Shiino, Watanabe, Maeda, Kotanin, Akiguchi & Matsuda 2006; Liang et al. 2008; Bai et al., 2011) in studies of atrophy (Jones et al., 2006) hypometabolism, (Minoshima et al., 1997), amyloid deposition (Frisoni et al., 2009), activation (Lustig et al., 2003), functional connectivity (Greicius et al., 2004) and with FC cognitive correlates in the present review.

Disconnection between the PCC and PCu was frequently associated with episodic memory retrieval and may reflect a reliable alteration in disease progression (He et al., 2007; Bai et al., 2008; Bai et al., 2009; Qi et al., 2010). Apart from episodic memory, connectivity between these regions is thought to subserve self reference (Fox & Raichle, 2007; Greicius, et al., 2004) internal mentation, functions of self, awareness and personal memory (Andrews-Hana, Reidler, Huang & Buckner, 2010; Buckner & Carroll 2007), all of which have been found compromised in persons with MCI and may be amongst the earliest signifiers of underlying pathology. These more subtle cognitive alterations would not have been directly detected by the more cognitive based tests included in the studies under review but such subjective contents which will likely vary as a function of episodic memory integrity.

The connectivity between the PCC and key nodes of the DMN including frontal, parietal and temporal regions connectivity again reflects the predominantly episodic memory deficits in patients with MCI, the lower connectivity between these regions appears to be related to early cognitive deficits. The regions correlated with delayed and immediate memory were remarkably convergent and attest to the role of DMN in episodic retrieval and encoding. The association with

medial parietal disconnection and cognitive impairment (Wang et al., 2012; Binnewijzend et al.,2012; Zhang et al., 2012) in MCI is in line with the sites of early amyloid deposition (Buckner et al., 2009) and metabolism abnormality (Sperling et al., 2010) described in AD. This region has also been shown to be compromised in task related imaging studies of AD (Petrella, Prince, Wang, Hellegers, & Doraiswamy, 2007).

The finding of hippocampal involvement in delayed and immediate memory is not surprising but the connection with the PCC suggests decreased cooperation between medial temporal lobes and PCC are a meaningful index for the earliest symptoms of cognitive decline in the memory domain. The association of hippocampal DMN connectivity and measures of episodic memory is functionally and anatomically in line with the pathogenesis and clinical manifestations of memory decline. It is also in line with that fact that hippocampal atrophy is one of the earliest indications of MCI and AD. However it appears the functional changes mediate cognitive alterations before structural deterioration is present because these relationships survive grey matter correction (Zhang et al., 2012). Findings related to thalamic functional connectivity may reflect a more general mechanisms of a large scale distributed network which supports memory function. Thalamic abnormality may mediate decreased connectivity of the key hubs of the DMN (Jones, Mateen, Lucchinetti, Jack & Welker, 2011) perhaps through decreased communication with the PCC (Vogt & Laureys 2005).

Associations between the DMN and cognitive function were only found to be relevant for other cognitive domains in one study (Binnewijzend et al., 2012). It is interesting that tests which measure such varied functions (visuospatial processing and memory, semantic and episodic memory and executive functions) were associated with lower FC of PCu, cuneus and PCC in the DMN. This may reflect the well known functions of these regions in multi-modal processing and integration (Cavanna & Trimble, 2006) and anatomical and functional connections to the hippocampus and functional links with the PFC and thalamus (Bai et al., 2009) may subserve these

relationships. It is surprising and limiting that only three studies assessed the resting state correlates of semantic memory as this has been found to be more sensitive and specific to early neurodegeneration than episodic memory (Gardini et al., 2013).

Taken together the findings suggest rather than reliance on distinct neural regions, higher order cognition requires large scale neural circuits to interact dynamically (Mesulam, 2009). Beyond the DMN, connectivity between other important regions was also reflected in variance in memory performance in MCI and across diagnostic groups. The centrality of the angular gyrus to episodic memory encoding and retrieval was suggested by several studies, this may be due to its multifunctional role in language and memory retrieval (Gardini et al., 2013). The FG is also frequently identified as a key component of memory circuits in the resting state (Buckner 2004) and several studies found dysfunctional FG connectivity was associated with episodic memory in the current review. The FG is reliably associated with memory operations especially recognition memory (McCarthy, Puce, Gore & Allison, 1997). The effect on the FG could also suggest a cascading effect of medial temporal lobe damage due to its communications with the hippocampus (Preston & Wagner 2007). As these regions are highly relevant for semantic memory (Binder, Desai, Graves, Conant, 2009) their association with episodic measures may also reflect significant shared variance between these domains.

The anatomical and functional relationships of the amygdala may explain its functional relationship with cognition. The amygdala is implicated in attention, perception and declarative, explicit and emotional memory (LeDoux, 2007) and mediates memory processes in brain structures such as the hippocampus and PFC (LaBar & Cabeza, 2006) it is also well connected to sensory areas. The insula also has important anatomical connections with key cortical, limbic and paralimbic regions and is, like the amygdala, well placed to mediate numerous functions from higher order cognitive ability, emotion to autonomic and sensory processes (Allen, Emmorey, Bruss & Damasio, 2008). The insula has also been associated with episodic memory (Fletcher, Shallice,

Frith, Frackowiack & Dolan, 1998) and it's connectivity with the right DLPFC is especially relevant for distributed mediation of episodic memory.

Executive function impairment can sometimes be associated with early MCI. The trends of positive correlation for executive function that lost statistical power after controlling for grey matter atrophy may be cautiously interpreted to be in line with the relationship between frontal-striatal-thalamic disconnection and issues with executive function frequently described by structural and functional imaging studies (Alvarez & Emory, 2006).

All correlations fundamentally reflected that abnormality in RSN connectivity was linearly associated with impaired cognition. The few inverse correlations reflected times when higher scores indicated higher impairment or higher network abnormalities. Whilst several studies found evidence for compensation, denoted by increased connectivity between certain regions in persons with MCI or AD most frequently in prefrontal regions, such increases were not directly associated with cognitive performance. As increases in connectivity may reflect compensation for impairment it is not surprising that these relationships are not detected in the general linear model and the vulnerability of the cognitive functions fostered by these increases will only become evident as a baseline impairment when the compensations mechanisms fails. This process was illustrated in the longitudinal study of Bai et al. (2011).

Conclusions

Frequently, lack of statistical power hampered the extent to which findings can be interpreted with confidence and more studies with larger groups are required in order for effect sizes to survive and statistical rigour to be preserved. Nevertheless, the current review suggests the findings of resting state studies of MCI patients are clinically meaningful.

Whilst methodological limitations are associated with all studies, these exploratory findings offer insight into neural mechanisms which subserve the episodic memory impairment that

characterises MCI. This suggests impaired anatomical functional integration impacts on memory performance in the early course of pathogenesis. Understanding more about the mechanisms that subserve the neuroanatomical and functional connections with aspects of cognition that mark disease progression is key to understanding how to improve early detection of MCI, mechanisms of conversion to AD and how and where to target treatment. More rigorous longitudinal studies are needed to investigate whether these functional and cognitive correlates are indeed related to AD progression. Further investigation of semantic memory in this context is a clear priority and all relationships should be regressed against the full-scale variance of the whole brain not extracted z scores.

Relationships, primarily between DMN especially the PCC, and episodic memory, tentatively suggest the clinical relevance of the DMN as a substrate that devolves in disease in a clinically relevant manner. Its involvement in episodic memory retrieval and encoding suggests it is an important marker of network pathology and cognitive decline. Therefore neuropsychological assessments, devised to assess the integrity of the DMN may improve dissociation of organic and functional episodic memory impairments. Based on what is known about the optimal function of this network such assessments are likely to include features of mentalisation, theory of mind and more ecologically valid measures of episodic memory such as autobiographical memory. In addition, this finding also suggests neuropsychological interventions such as cognitive stimulation and neurofeedback should be targeted to reinforce connectivity between substrates in the DMN. Additionally, the DMN could represent a valuable biomarker for assessing outcomes of neuropsychological and pharmacological interventions.

Apart from episodic memory, the DMN supports a range of important functions such as autobiographical memory, meta-cognition, theory of mind, self reference etc. which have been found to be vulnerable in prodromal AD pathology but are typically not included in neuropsychological assessment of persons with possible MCI. Dysfunction of a network supporting

such processes may explain the subjective experience of MCI as a partial disconnection from or loss of self (Clare et al., 2012), unfortunately such features are infrequently investigated and often seen as emotional sequelae of memory anxiety. However, they may reflect the early disruption of a network which subserves personal memory and representations of self and other. In light of this, neuropsychological measures could be developed to be more sensitive and specific to the range of cognitive and emotional functions supported by this network. This behavioural relevance of this network may therefore play an important role in early detection, predicting progression, targeting behavioural and pharmacological amelioration and measuring outcomes.

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Section 2: Research Report

The neuropsychological correlates of posterior cingulate resting state connectivity in normal and abnormal ageing

Table of Abbreviations

AD = Alzheimer's disease, MCI = Mild Cognitive Impairment, OHC = Older Healthy Controls, YHC = Younger Healthy Controls, fMRI = Functional Magnetic Resonance Imaging, MRI = Magnetic Resonance Imaging, RSN = Resting State Network, DMN = Default Mode Network, FC = Functional Connectivity, ICA = Independent Component Analysis, ROI = Region of Interest, PFC = Prefrontal Cortex, PCC = Posterior Cingulate Cortex, MMSE = Mini Mental State Examination, SPM = Statistical Parametric Mapping, FDR = False Discovery Rate, BA = Brodmann area, L = Left, R = Right.

The neuropsychological correlates of posterior cingulate resting state connectivity in normal and abnormal ageing

Objectives. The posterior cingulate cortex (PCC) plays a central role in episodic memory, may subserve some semantic memory functions and is considered one of the earliest imaging markers of Alzheimer disease (AD) pathology. The objective of this thesis was to ascertain if clinical variance in semantic and episodic memory is associated with disrupted connectivity of the PCC in Mild Cognitive Impairment (MCI) in comparison with age matched and younger controls to ascertain whether PCC associations with semantic and episodic memory are modulated by normal and abnormal ageing.

Design and methods. In this cross sectional study, resting state functional magnetic resonance imaging was used to acquire functional brain images for 22 patients with MCI, 21 age matched healthy controls and 20 younger healthy controls. PCC regions of interest (ROI) were defined for each participant and separate multiple linear regressions were computed for each group to detect associations between the whole brain connectivity of right and left PCC ROIs and measures of episodic and semantic memory performance.

Results. Behaviourally, episodic scores were sensitive to the effects of abnormal ageing however semantic scores were both sensitive and specific as they were robust to normal ageing. Inverse associations were evident for PCC connectivity with semantic and episodic memory for age matched controls, whereas positive correlations were demonstrated with just the episodic index and PCC connectivity for MCI patients and younger controls. As expected, MCI was associated with lower connectivity between the PCC and posterior default network regions which subserve memory functions whilst connections between the PCC and posterior memory regions were maintained in normal ageing.

Conclusions. The results suggest the episodic correlates of PCC connectivity are meaningful biomarkers of normal and abnormal ageing. The association with MCI is in line with hypotheses of the early episodic memory impairment being associated with reduced connectivity between the PCC and posterior default mode network (DMN) regions. In normal ageing the negative correlates of episodic memory and PCC connectivity may be in line with cognitive efficiency and frontal theories of ageing.

Practitioner points

- The results suggest, alongside episodic memory assessment, measurement of semantic memory is important for early detection of abnormal ageing
- The anatomical, functional and physiological meaning of the relationship between the PCC and episodic memory suggest a biomarker for cognitive assessment, longitudinal follow-up and for measuring treatment outcomes
- The relevance of including further DMN relevant measures such as autobiographical memory or indications of self reference for assessment purposes should be investigated in future work
- Future studies with larger samples are needed to explore these results in a higher powered context
- Due to the earliness, sensitivity and specificity of semantic memory to abnormal ageing studies with combined analyses methods are needed to ascertain whether variance in semantic memory is reliably associated with other nodes and resting state networks

Neuropsychology in the context of normal and abnormal ageing is concerned with understanding the way in which both physiological and psychological factors interact to result in cognitive performance (Woodruff-Pak, 1997). In order to understand normal and abnormal ageing in meaningful ways, physiological and psychological function must be assessed.

Amongst the multidisciplinary professions that have converged to further an understanding of normal and pathological ageing, neuropsychology continues to be the forerunner in the clinical assessment and detection of possible neurodegeneration (Weintraub, Wicklund & Salmon, 2012). In the continuing search for biomarkers it is easy to forget that as cognitive symptoms are always the first indication of possible underlying neuropathology, emerging cognitive impairment continues to reflect the most important marker of disease (Weintraub et al., 2012). However, cognitive symptoms are only of diagnostic meaning in the context of what they may imply about the integrity of the neural mechanisms which are known to subserve that aspect of cognition (Venneri et al., 2011). It is therefore the interface of brain and behaviour that reflects the most salient markers of health and illness. This interface forms the basis of neuropsychological assessment to identify when altered memory reflects neuropsychological impairment and whether this deficit is mediated by functional or organic aetiology (Morris, Worsely & Matthews, 2000).

Alzheimer's disease (AD) is the most common neurodegenerative disorder and it increasingly represents one of the most important and concerning public health issues (Nestor, Sheltons & Hodges, 2004). The emotional and financial costs of this disease are personally and systemically devastating and as the ageing population increases, the incidence of this disease is predicted to rise dramatically (Cummings, 2003). The effects of AD are irreversible and inevitably AD neurodegeneration results in extensive damage of the cerebral cortex. Progression of this damage is clinically manifest as increasing cognitive impairments and behavioural disturbances (Lopez & Becker, 2004). In the absence of a cure for this disease, the most promising areas of intervention are neuropsychologically (Jelcic et al., 2012; Sitzer, Twamley & Jeste, 2006; Olazaran et al., 2010) or pharmacologically based (Birks, 2010). However, due to the irreversible neural damage caused by the pathogenesis of AD, intervention needs to be undertaken as early in the course of disease as possible (Wang et al., 2012).

Mild cognitive impairment (MCI) is thought to reflect incipient AD pathology (Petersen, 2001). As MCI has been characterised as a transitional phase residing between normal ageing and dementia of the Alzheimer type it represents an important target for early treatment (Petersen, 2009). However, individuals with MCI only convert to AD if they have underlying AD pathology (Dubois et al., 2010). Individuals with MCI that is not mediated by AD may improve, remain stable or progress to a neurodegenerative disorder of another aetiology (Petersen et al., 2001). It is therefore difficult to identify the persons who would benefit from the cognitive or pharmacological treatments that are specifically targeted for the brain regions and networks that are most vulnerable to AD pathology and progression. Early pharmacological interventions have already proven beneficial for some persons with AD (Birks, 2010). However, these medications may be ineffective or may even worsen the symptoms when administered to patients with no underlying organic pathology or whose MCI is related to other dementia pathologies (Balster et al., 2011).

The most promising line of recent research suggests that specific non pharmacological behavioural interventions such as cognitive stimulation (Sitzer, et al., 2006; Olazaran et al., 2010) may slow the progression or the onset of AD. However, such interventions need to be based on the neuropsychological function of the brain regions that are involved in the cognitive symptoms of AD and MCI and their effectiveness must also be assessed in relation to relevant neurobiological as well as cognitive markers (DeMarco et al., 2012). Stability or improvement in neuropsychological scores alone may not be sufficient evidence that cognitive stimulation programmes are effective, without comparing a relevant hypothesis driven brain structure or network at baseline and follow-up, any changes in behavioural data could merely reflect practice effects or compensation.

Compensation can mask the extent of pathogenesis and may lead to an underestimation of progression (Bai et al., 2011). An individual whose neuropsychological profile suggests signs of early MCI may have much further progressed neuropathology if they have the cerebral reserve or plasticity to compensate for cognitive alterations. Compensation refers to a process whereby

damage in cognitive circuits leads to cerebral reorganisation of function (Kolb, 1995). When an individual compensates in response to loss or impairment, they must be using available brain substrate and mechanisms which allow accommodation to structural (Kolb, 1995) or functional (Bai et al., 2011) loss which can preserve or adapt behavioural function. However, this has important implications for early detection and diagnosis of neurodegenerative disorders and for measuring intervention outcomes. If an individual is compensating neurologically, the underlying impairments may not be evident or fully expressed in behavioural performance (Staff et al., 2004). In such cases, combined neuropsychological and neuroimaging methods are required to identify the extent to which neuropsychological markers are in line with the underlying disease process.

Whilst MCI previously represented an important at-risk status for clinical follow-up and monitoring of possible progression (Petersen et al., 2001), now viable treatment options are available it is increasingly important to identify persons with MCI whose symptoms are related to incipient AD pathology (Agosta et al., 2012). Whilst there are now many reliable candidate genetic, histological, radiological and imaging markers of AD, such as the apoliopoprotein E ε4 allele, medial temporal lobe atrophy, temporo-parietal hypometabolism, abnormal amyloid or tau cerebral spinal fluid concentration (Dubois et al., 2010) which may be identified in persons with MCI (Agosta et al., 2012), these markers are rarely assessed in clinical settings and are often associated with or not detected until cognitive impairment is more severe. Moreover, once certain markers such as regional atrophy are clearly detectable in the brain on an individual level, the damage may be too progressive for treatment to be effective. Whilst cognitive impairment reflects damage to the tissue that supports that function (Rodriguez-Ferreiro, et al., 2012), certain neuropsychological alterations appear to precede structural atrophy (Bai et al., 2011; Binnewijzend et al., 2012), and this context presents an ideal treatment opportunity, especially if these early changes are associated with functional markers of AD pathology.

Episodic Memory

In line with a transition to AD, the earliest and most frequent clinical feature of MCI is typically an impairment of episodic memory (Collie & Maruff, 2000); this is especially evident in the impaired learning of new episodic memories (Fox, Warrington, Seiffer, Agnew & Rossor, 1998). Such deficits are consistent with the established relationship between the anterograde amnesia with the hippocampus and entorhinal cortex and the well known vulnerability of these regions to very early AD pathogenesis (Rodriguez-Ferreiro et al., 2012).

In terms of the neuropsychological markers which may be indicative of MCI, measures of verbal episodic memory tests appear to be most sensitive to early change. Consistency in performance across a range of episodic memory measures has been implied as essential to defining amnestic MCI (Lonie, Hermann, Donaghey & Ebmeier, 2008). Episodic memory impairment has been identified as the most reliable predictor of conversion to AD (Mitchell, Arnold, Dawson, Nestor, & Hodges, 2009) and episodic memory tests can contribute to differential diagnosis of MCI (Irish, Lawlor, Cohen & O'Mara, 2011). Delayed recall paradigms have been noted as important assessment modes to discriminate individuals with MCI who may be most vulnerable to convert to AD (Backman, Jones, Berger, Laukka, & Small, 2005; Dubois & Albert, 2004). Detailed longitudinal evaluation of neuropsychological performance in MCI and questionable dementia suggests visuospatial functions tend to fail secondarily to episodic memory (Hodges, Erzinclioglu & Patterson., 2006). In addition, processing speed impairments may be more indicative of sub cortical or cerebrovascular pathology (Zhou & Jia, 2009) and tests of attention and spatial and temporal orientation are also thought to be too susceptible to the effects of normal ageing to reliably discriminate MCI from healthy controls (Gardini et al., 2013).

However, whilst neuropsychological assessment of episodic memory impairment is sensitive to pathogenesis it is not specific to this, episodic memory alterations can also be associated

with normal ageing, functional disorders such as depression or with non AD pathology.

Semantic Memory

In addition to episodic memory, semantic memory impairment may be an equally important clinical feature of MCI (Cuetos, Rodriguez-Fernando & Menendez, 2009; Venneri et al., 2011). Semantic memory refers to culturally shared knowledge that is acquired over a lifetime; it includes knowledge about the world, people and objects, word meanings and facts (Gardini et al., 2013). Unlike episodic memory, semantic memory is more temporally stable across the lifespan, robust to the effects of ageing (Venneri et al., 2011) and sensitive to neurodegeneration (Venneri et al., 2011b). Whilst semantic memory impairments have been associated with the development and progression of dementia (Venneri, et al., 2011) as deterioration in semantic memory precedes dementia onset by many years (Gardini et al., 2013) it also represents one of the earliest signs of cognitive impairment (Amieva et al., 2008). Tasks such as confrontation naming, semantic associations and category fluency have been used to detect semantic memory impairment in persons with MCI (Duong, Whitehead, Hanratty & Chertkow, 2006). Verbal fluency for categories has been found to discriminate between persons with MCI and age matched controls (Nutter-Upham et al., 2008).

However, whilst semantic memory impairment may be equally sensitive and more specific than episodic memory impairment in discriminating MCI from healthy older adults, increasing evidence suggests that rather than using a unimodal strategy to assess and diagnose MCI, there is great clinical advantage to be gained from using a combined neuropsychological and neuroimaging approach (Venneri et al., 2011). Combining these methods will also allow evidence of compensation to be assessed. Furthermore, such a multidisciplinary approach is especially necessary to improve detection of individuals with MCI who may be at risk for conversion into AD (Venneri et al., 2011).

Resting State Imaging Biomarkers

Due to the shift in focus onto early detection and the need to find sensitive ways to assess treatment efficacy (Matsuda, 2007), biomarkers, as identified by neuroimaging methods, have become increasingly important in improving diagnostic reliability (Zamrini, McGwin & Roseman, 2004). Originally, imaging techniques designed to understand complex relationships between the brain and behaviour in health and illness were solely focused on detecting neural activation with task performance. However, recently the relevance of regions that deactivate during tasks and the spontaneous neural activity observed when an individual is at rest have become a major focus of attention (Greicius et al., 2004).

Resting-state describes a situation in which the activity of the brain is imaged in the absence of external stimulation. The participant simply lies still in the scanner with their eyes closed (Bai et al., 2009). In such studies, spontaneous correlations between and within regions of the brain can be characterized in an individual who is not engaged in a cognitive task or motoric response to a task (Damoiseux et al., 2007). Synchronized neural activity can thus be detected between regions that are spatially separate but temporally associated (Esposito et al., 2006).

Task free resting states may be equally or even more important for understanding the mechanisms which subserve health and disease than task induced neural systems (Broyd et al., 2008). It is now apparent that when the brain is not engaged with external tasks, cerebral activity is coherently organised in different dynamic subsystems (Damoiseaux et al., 2008). In persons at rest, low-frequency spontaneous correlations are detectable across the brain with functional magnetic resonance imaging (fMRI). These correlations can be used to characterise the intrinsic architecture of large-scale brain systems. It is especially salient that the temporal organisation of these subsystems closely resembles structurally and functionally related neurophysiological and neuropsychological networks such as the motor cortex (Biswal, 1995), visual cortex, language

network, dorsal and ventral attention streams (Damoiseaux et al., 2006; Fox et al., 2006). These systemic connectivity data have contributed to an understanding of normal and pathological brain function (Buckner et al., 2008) as resting-state networks (RSNs) are thought to be intrinsically important to the organisation and maintenance of functional neural relationships, alterations to these networks will clearly have important clinical implications (Damoiseux, et al., 2007). Several studies have demonstrated that investigation of RSNs can reveal important information in clinical contexts suggesting the study of spontaneous brain activity could contribute to explanations of disease (Buckner, Andrews-Hanna & Schacter, 2008).

The Default Mode Network

The most recent and perhaps the most promising neuroimaging biomarker to be investigated in this context is the default mode network (DMN) of the brain (Fox & Raichle, 2007). This network was originally noticed in the context of task related imaging studies, whereby despite the cognitive modality of a task, and the areas of activation induced by response to a paradigm, the DMN network was reliably deactivated (Binder et al., 1999; Shulman et al., 1997). Comparison of this consistent and robust finding across many studies led to the definition of this network (Greicius et al., 2003). The deactivation of the DMN in response to external tasks and its reliable appearance in resting state studies when an individual has no task, led to the concept that the DMN reflects key internal modes of cognition (Damoiseux et al., 2007). For such reasons, the DMN network is thought to reflect the universal baseline cognitive state. The clinical importance of this system, which is still somewhat novel, has only recently been appreciated (Buckner et al., 2008).

It has been suggested that the source of the default mode of the brain lies in the spontaneous generation and manipulation of mental images, reminiscences of past experiences based on episodic memory, making plans, self reflection, self reference, mind wandering, theory of mind and automatic monitoring (Spreng, Mar & Kim, 2009; Spreng & Grady, 2010). The DMN is an

independent neurobiological system which represents a brain system (or closely interacting subsystems) involving anatomically connected and interacting brain areas. Anatomically the DMN involves the posterior cingulate cortex (PCC), precuneus, dorsal and ventral prefrontal cortex, medial temporal lobes and sometimes the thalamus (Greicius et al., 2003; 2004). The known functions of these anatomical substrates explain why the DMN mediates the above stated episodic memory, autobiographical and referential processes (Greicius et al., 2004; Spreng & Grady, 2010). The cognitive and emotional functions supported by substrates in this network further attest to the importance of the DMN in understanding relationships between personal memory, the self and the brain in health and illness (Bucker et al., 2008).

In the healthy brain, changes in activity levels in the DMN are detected when an individual is engaged in an active cognitive task in normal ageing (Grady, Springer, Hongwanishkul, McIntosh, & Winocur, 2006) in states of meditation (Jang et al., 2011), hypnosis (McGeown, Mazzoni, Venneri & Kirsch, 2007) and anaesthesia (Deshpande, Kerssens, Sebel & Hu, 2010; Nallasamy & Tsao 2011). Moreover, such alterations have been associated with diseases including neurodegenerative diseases such as Alzheimer's disease (Greicius, Srivastava, Reiss, & Menon, 2004), Lewy Body dementia (Galvin, Price, Yan & Morris, 2011) and Parkinson's disease (van Eimeren, Monchi, Ballanger & Strafella, 2009), developmental and psychiatric disorders such as schizophrenia (Garrity et al., 2007) and autism (Iacoboni, 2006) and psychiatric diagnoses such as obsessive compulsive disorder (Fitzgerald et al., 2010), depression (Sheline, Price, Yan & Mintun, 2010) and substance misuse.

It is of note that in normal ageing the DMN has been found to be less efficient at deactivating during tasks in fMRI conditions and this reduced deactivation has been associated with lower task performance (Grady et al., 2006). In abnormal ageing, individual circuits in the default network appear to be targeted by specific neurodegenerative diseases (Seeley et al., 2009) especially AD. DMN regions comprise the typical early predilection sites of AD (Mosconi, 2005)

and AD pathology appears to continue to progress preferentially in the DMN (Greicius et al., 2004). More specifically, decreases in activity within the DMN have been found to parallel increases in atrophy and map consistently with the regions of amyloid deposition in Alzheimer's disease especially in the medial temporal lobes and PCC (Greicius, et al., 2004). The considerable overlap between the DMN and the brain regions which subserve the cognitive functions that are found to be most vulnerable to deterioration in AD and MCI is in line with the hallmark episodic memory impairment. It is this overlap with cognition that suggests that rather than just reflecting a proxy for disease, DMN dysfunction is likely to be expressed in a cognitively meaningful impairment.

This evidence is in line with the 'Metabolism Hypothesis of Alzheimer's disease' (Buckner et al., 2005). This hypothesis suggests that the DMN would be a good predictor of neurodegenerative disease because AD pathology appears to aggregate preferentially within the network and this explains the neuropsychological sequelae of the disease. Prospective studies have demonstrated that non clinical individuals who later develop AD do not deactivate their DMN in the same manner as persons who are not at risk (Buckner et al., 2008). The hypothesis would suggest that for these persons these regions are overworked to the extent that their resistance to the neuropathological process is limited. This would also explain why episodic memory is so vulnerable to early AD and MCI because these systems are anatomically and functionally dependent on the resting state of the default network that is being overused. Thus amyloid beta could initiate a cascade of events beginning in synaptic dysfunction and ending in cell death within this essential network (Buckner et al., 2008). In this respect, impairments in the DMN may actually facilitate disease. Similarly, when an overextended, compensation mechanism in the DMN fails, the functional disruption and accelerated cognitive decline can be more dramatic than in those who do not compensate (Bai et al., 2011). Excessive DMN activity may therefore increase amyloid production (Bero et al., 2011) and be causal in disease progression.

Following the first study to identify that DMN alterations in the resting state distinguished persons with AD from age matched controls (Greicius et al., 2003), fMRI of DMN brain activity during rest has gained much attention as a potential non invasive biomarker to diagnose incipient AD and recent studies have consistently found DMN disruption in persons with MCI (Binnewijzend et al., 2012; Petrella, Sheldon, Prince, Calhoun & Doraiwamy, 2011; Sorg et al., 2007). Differences in DMN disruption have also been found between persons with stable MCI and those who later converted to AD (Petrella et al., 2011). DMN alteration has also been identified in persons carrying the most significant genetic factor of AD risk, the apoliopoprotein E ε4 allele (Westlye, Lundervold, Rootwelt, Lunderveld & Westlye, 2011) and persons with a family history of AD (Fleisher et al., 2009).

Very recently, several studies have combined this type of neuroimaging endophenotype with cognitive markers of prodromal AD and found that in persons with MCI, decreased connectivity in the DMN is associated with impaired cognition and as these relationships are independent of atrophy, they appear to suggest that functional alterations in the DMN is sufficient to mediate impaired cognition at an early stage of pathology. The previous thesis literature review investigated studies that explored the neuropsychological correlates of the DMN and other resting state networks. The predominant finding suggested the episodic memory impairment in persons with MCI is linearly dependent on the integrity of connections between medial parietal, temporal and frontal regions of the DMN. Episodic memory performance appears to decrease as a function of decreased coherence of the spontaneous correlations between these regions. The most consistent finding was that decreased connectivity between the PCC and other DMN regions appeared to be the most robust correlate of episodic memory impairment in persons with MCI.

Posterior Cingulate Cortex

The PCC is an important part of the DMN, it has been identified as a functional and structural core of this network (Fransson & Marrelec, 2008; Honey et al., 2007). It is also proposed

to be one of the most important regions in the pathogenesis of AD and MCI (Bai et al., 2009; 2011). According to studies of histopathology (Rowe et al., 2007), structural integrity (Shiino et al., 2006), functional imaging studies (Liang et al., 2008) and resting state studies of AD (He et al., 2007; Greicius et al., 2004) and MCI (Bai et al., 2008) PCC abnormality is central to pathology of the AD type across the continuum of disease progression (Bai et al., 2009).

The PCC may reflect a relevant marker for neuropsychological and neurological impairment (Bai et al., 2009). The associations between the PCC and impaired episodic memory and decreased connectivity in the DMN suggest potential for a combined brain behaviour biomarker for incipient AD.

Whilst the known brain behaviour relationships of the DMN attest to its importance for episodic memory, several regions of the DMN have also been associated with semantic memory in structural and task based functional imaging studies (Binder, Desai, Graves & Conant, 2009). Similarly, several of the main functions associated with the DMN such as episodic memory, autobiographical memory and internal reflections of self and other are likely to rely to some extent on a semantic store and will therefore share a level of variance with aspects of semantic memory. Whilst several studies have assessed the structural substrates of semantic memory impairment in MCI (Gardini et al., 2013; Rodriguez-Ferreiro et al., 2012) there is little known about whether variance in semantic memory is associated with functional connectivity in the resting state.

Whilst no study has formulated a specific hypothesis about resting state connectivity and semantic memory, a limited number have included measures of semantic memory in correlations with resting state network connectivity (Agosta et al., 2012; Jin et al., 2012 and Binnewijzend et al., 2012). Whilst Agosta et al. (2012) and Jin et al. (2012) found no relationships between their measures of semantic memory and resting state connectivity, Binnewijzend et al. (2012) found that impaired category fluency scores were positively associated with lower connectivity between the

PCC and precuneus of the DMN. These studies were underpowered and only looked for behavioural associations as a secondary aim. The lack of hypotheses led to the inclusion of numerous neuropsychological tests, which in combination with small samples meant that findings must be cautiously interpreted. Additionally, Jin et al. (2012) used a measure of semantic association which may be a less sensitive and specific measure than tests such as category fluency (Gardini et al., 2012).

A recent meta-analysis, across one hundred and twenty functional imaging studies, revealed semantic memory relies on a network of brain regions. The three main neural circuits associated with semantic memory storage and retrieval included structures that are either part of the DMN or highly functionally and/or anatomically connected with this network (Binder et al., 2009). The heteromodal frontal network included dorsal and ventromedial prefrontal cortex (PFC), a paralimbic circuit included regions with connections to the parahippocampus and PCC and the posterior heteromodal association cortex included the angular gyrus, middle temporal gyrus and the fusiform gryrus (Binder et al., 2009). Whilst the dorsal and ventromedial PFC and connections of the paralimbic circuit with the PCC and parahippocampus are intrinsic to the DMN, the previous thesis literature review revealed that disrupted DMN connectivity with structures in the association cortex are also associated with impaired episodic memory. Decreased DMN connectivity or lower network strength between the angular gyrus and middle temporal gyrus was associated with episodic memory impairment (Jin, Pelak & Cordes, 2012; Wang et al., 2013) and an association between decreased connectivity between the precuneus and angular gyrus and episodic memory impairment approached significance in two further studies (Jin et al., 2012; Liang, Wang, Yang & Li, 2012). The strength of causal influence exerted from the middle temporal gyrus to the fusiform gyrus was also positively associated with episodic memory (Yan et al., 2013) and a trend for decreased fusiform connectivity in DMN was nearly significantly associated with impaired episodic memory (Jin et al., 2013). As these regions are more robustly associated with semantic than

episodic memory, these correlations may be detecting a degree of overlap between episodic and semantic memory associated or varying with measures of episodic memory. Additionally, one may expect a level of shared variance between regions that support episodic memory and internal mentation with semantic memory. However, whatever explanation, the fact that explorations of behavioural correlates of DMN connectivity was able to detect associations between the DMN and regions which typically subserve semantic memory suggests the anatomical and functional breadth of DMN connectivity ought to detect theoretically and clinically meaningful functional correlates of semantic memory.

Whilst the PCC connectivity with the DMN appear highly sensitive to episodic memory, PCC relationships with non DMN structures may also reveal other relevant relationships that may explain the earliness and the sensitivity and specificity of the semantic memory marker of neurodegeneration.

Aims, Hypotheses and Research Questions

The aims of the proposed study are to characterise the way in which PCC connectivity is reflected in indices of cognitive performance which are associated with early MCI. Understanding how the PCC relates to behavioural markers of pathology such as cognitive performance will inform the clinical utility of pursuing these brain behaviour relationships as markers of MCI with AD pathology. Using a hypothesis driven approach to the neuropsychological tests used should also limit the problem of multiple comparisons which confounded more exploratory studies of behavioural correlates of resting state networks. Index scores will be used to reduce random variability and limit floor and ceiling effects.

Brain regions are not discrete units, they participate dynamically to create highly interconnected cerebral networks (Bai et al., 2009) and in order to be clinically meaningful the relevance of the connectivity between regions of these networks for cognition should be explored

(Venneri et al., 2011). Therefore, the purpose of the current study was to investigate the relationship between resting state whole brain connections of the PCC and the two most valid neuropsychological markers of incipient AD, episodic and semantic memory across and between groups of persons with MCI, an age matched control group and a young control group. This is therefore the first cross sectional study to employ a design that will be able to differentiate age related associations from those related to disease processes. It is also the first to regress behavioural and connectivity associations against the full-scale variance of the whole brain as opposed to statistically extracted z scores.

Based on the behavioural evidence, it is predicted that episodic scores will be sensitive to the difference between MCI and healthy age matched controls, but will not be specific to this as they will be susceptible to an ageing effect as young healthy controls will obtain significantly higher scores than older healthy controls. Whereas semantic memory is only predicted to differ between MCI and age matched controls as it is not thought to be susceptible to the effects of normal ageing.

Due to the predicted disruption of the hippocampus with the DMN, the effect of disease is hypothesised to be reflected in lower PCC connectivity with anterior memory structures. It is expected that disrupted functional connectivity between the PCC and medial temporal and medial parietal lobes will be evident in persons with MCI compared with healthy age matched controls. Connectivity between the PCC DMN is not predicted to be significantly different between healthy older adults and younger individuals although the older group may demonstrate some signs of cognitive efficiency (Sperling et al., 2009).

Within the separate MCI group, lower PCC connectivity with regions of the posterior DMN is predicted to be associated with episodic memory impairment. Further research questions pertain to whether PCC connectivity will be associated with semantic memory in MCI or control groups.

Method

Design

The data in the current study are secondary, and were obtained from a database that is currently under development in a department of clinical neuroscience within a private hospital in Northern Italy. The database includes pooled demographic, physiological, neuroimaging, genetic and neuropsychological data for both patients and controls. The patient data has been collected through routine clinical diagnostic work and clinical research, the participant data has been provided by a group of hospital staff and local volunteers who have specifically volunteered to provide norms and controls for clinical standardisation and future research. Additionally, as the data used in the current thesis are secondary, an explanation of the author's personal role in the design of the study and analyses can be found in Appendix 2. The cross sectional design allowed hypotheses to be investigated about whether the effects of abnormal (MCI compared to OHC) and normal ageing (OHC compared to YHC) modulate the relationship between regional PCC connectivity and episodic and semantic memory.

Power analysis

Due to the recent emergence of this area, it was not possible to conduct a power analysis. Consequently, sample size was based on the numbers used in the empirical papers included in the thesis literature review. In this literature review, significant relationships were found in studies with as few as eight participants per group to a maximum of 35. Without knowing the effect sizes of these relationships, an average of 20 participants per group appears to be consistent with good quality studies in this area.

Participants

Out of an original 71 participants a final total of 63 were included in this study, all were

recruited from the San Camillo Hospital, Lido, Venice. The neuroimaging and neuropsychological data for the MCI group was baseline data of an earlier intervention study (DeMarco et al., 2012) which had been added to the research database described above. Under Italian law apart from study specific consent forms all participants must complete a general consent form. This form includes a section whereby participants can make informed consent for their data to be used in future research. All the patient and control participants included in this retrospective study had signed this section of the general consent form. The data for the age matched healthy controls and for the young healthy control groups was obtained from the research database that is being developed at San Camillo. This database is comprised of hospital staff and friends and relatives of patients. These individuals provided consent for their neuroimaging and neuropsychological data to be placed on the database for use in future clinical research studies on the same general consent form. A copy of this form can be found in Appendix 7.

All participants underwent diagnostic evaluation, clinical interview and demographic inventory. Exclusion criteria included history of stroke, cardiovascular disease, substance misuse, psychotropic medicine, head injury, Parkinson's disease, other neurological or psychiatric illness, epilepsy or current major medical illness. No participant was excluded on the basis of these criteria. The T2 weighted images were screened for signs of cerebrovascular disease and other signs of brain disease including lacunae. Three MCI and two older healthy controls (OHCs) were excluded due to extensive white matter changes.

Twenty two MCI patients (mean age 75.33, *SD* 6.96, mean education 9.95, *SD* 4.21, 8 males) were assessed by experienced clinical neuropsychologists. All patients met published criteria for MCI of the amnestic type (Petersen, et al., 2001). These patients had no evidence of dementia, no deficits in other cognitive domains and intact daily living skills. In line with Petersen (2001) criteria, three patients with Mini Mental State Examination (MMSE: Folstein, Folstein & Mchugh, 1989) less than 24 were excluded from the study. All controls were required to have MMSE of 28 or

higher, no participant was excluded on this basis. As the twenty one OHCs (mean age 66.36, *SD* 7.45, mean education 10.86, *SD* 4.89, 11 males), were significantly younger than the MCI patients, age was controlled in all analyses. The young healthy control group (YHC) was comprised of twenty people (mean age 38.25, *SD* 8.24, mean education 16, *SD* 3.23, 8 males) as education was significantly different between the YHC and MCI and YHC and OHC, it was included as a covariate in all models.

Ethical Approval

Ethical approval for the retrospective use of patient and control data was granted by San Camillo Hospital, Lido, Venice, Italy. A copy of this letter can be found in Appendix 3. Scientific approval was granted by the department of Clinical Psychology, University of Sheffield and Clinical Governance was sponsored by the University of Sheffield Copies of these letters can be found in Appendices 5 and 6.

Procedure

Although the data was obtained from a previous clinical study and participant database, the procedure was uniform across all participants.

Neuropsychological Assessment

Experienced clinical psychologists undertook the neuropsychological assessment with patients and controls. Each participant had undertaken the same battery of neuropsychological tests comprised of cognitive tests which are routinely used for diagnosis of neurodegenerative disorders in clinical practice. As the battery is part of routine clinical practice, an active research database and an extensive standardisation study, certain test may not be the most recent version. However, each test within this battery is known to be a reliable and valid measure of the domain for which it was intended to assess and in order to circumvent the Flynn effect the original norms are not utilised. In

the current study, all scores were standardised against the mean scores of the young healthy control group. The MMSE was used to assess disease severity through global cognitive functioning. Episodic and semantic tests were chosen from the patients and participants' batteries.

Episodic Index Score. An episodic memory index score was derived from the following tests of episodic memory. Logical Memory Immediate and Delayed Recall (Wechsler, 1997a), in this test individuals are read a short story and asked to recall as much as possible immediately after it was read and after a ten minute delay; answers are scored as individual story items and thematically. Rey Complex Figure - Delayed recall (Osterreith, 1944), the participant is shown a picture of a complex figure and asked to copy it, for the delayed scores, participants are asked to reproduce the figure after a ten minute delay. Verbal Paired Associates (Wechsler, 1997a) is a test of verbal learning; participants are presented with the same eight word pairs in different orders over three trials, half of the words are easily paired, the other half are more difficult. The score is the total number of pairs learnt over the three trials. In the Corsi Visual Span test (De Renzi & Nichelli, 1975) the patient is presented with a board with nine 3D blocks, the examiner taps a sequence on these blocks and the participant is required to replicate this sequence, there are three trials for each sequence and participants are required to achieve two correct sequences before progressing. Visual Supraspan (Capitani, Gross, Lucca, Orsini & Spinnler, 1980) using the same apparatus, the examiner taps a complex sequence of nine blocks and the participant is asked to replicate this, this demonstration and replication continues for 18 trials or until the participant achieves two consecutive accurate trials.

Semantic Index Score. A composite score for semantic ability was derived from Confrontational Naming (unpublished) and Semantic Fluency (Lezak, Howieson, & Loring, 2004). In Confrontational Naming, participants are shown twenty line drawings and asked to name them (Snodgrass & Vanderwart, 1980). In the Semantic Fluency task participants are asked to name as many exemplars as possible from a given category in one minute, the categories used in the current

study were animals, fruits and clothing. Similarities (Wechsler, 1997b) scores were not included as they were not found to be significantly different between the MCI and OHC groups. Similarities is a test of semantic association and abstract verbal reasoning, in this task participants are given two items and are asked to identify the way in which they are similar, items progress from easily associated pairs such as fork and spoon to more abstract relationships such as poem and statue. The separate semantic and episodic index scores were obtained by using the mean and standard deviation of the YHC performance on each test as the normative baseline upon which each raw test score was converted to a Z score. The final composites were comprised through averaging the individual Z scores across each domain.

MRI Acquisition

Structural and resting state functional MRI images were acquired on the same1.5 Tesla Philips® Achieva MRI system. The three dimensional T1-weighted scan was acquired with a Turbo Field Echo sequence. The Voxel dimensions were 1.1 X 1.1 X 0.6 mm and field of view was 250 mm with a matrix size of 256 X 256 X 124. Total acquisition time was 4 minutes 27 seconds (repetition time, RT: 7.4 ms, echo delay time, TE: 3.4 ms and flip angle 8 degrees). For resting state scan echo planar T2* weighted MRI images were acquired on the same 1.5T Philips® Achieva system (TR=2s, TE=50ms, flip angle 90◦, voxel dimensions 2.00x2.00x3.00mm^3, field of view 230 mm). Two hundred and forty volumes of 20 contiguous axial slices were acquired in ascending order. Acquisition time lasted approximately 8 minutes. Prior to proper scans acquisition 20s of dummy scans were acquired to allow the scanner to reach equilibrium. During the scans, no specific instruction was given to the participants. They were only asked to remain as still as possible and with their eyes closed for the full duration of the scan and avoid any movements. Additionally a fluid-attenuated inversion recovery (FLAIR) coronal scan and an axial T2-weighted structural scan were also acquired to evaluate vascular burden in detail. A safety buzzer was provided in case participants required assistance.

MRI analysis

All postprocessing was undertaken by an experienced clinician. The MRIs were examined for abnormalities through visual inspection by this individual. The resting state fMRI preprocessing was undertaken with Statistical Parametric Mapping (SPM8) image analysis software (Wellcome Centre for Neuroimaging, London, UK). All volumes from each subject were re-aligned. After creating a mean as a reference they were subsequently re-sliced using 4th Degree B-Spline interpolation methods to adjust for residual motion related signal changes. Images were spatially normalized to the standard EPI template available in SPM8 using non-linear estimation of parameters. Normalized images were then spatially smoothed with an 8 mm full width at half maximum isotropic Gaussian kernel to compensate for any residual variability after spatial normalization. Image data were high-pass filtered with a set of discrete cosine basis functions with a cut-off period of 550 s. Additionally, head motion was included as a regressor in the first level analyses.

In order to co-vary for grey matter volume, the structural scans of each participant were preprocessed with SPM8. Segmentation of grey matter, white matter and cerebrospinal fluid allowed statistical computation of the proportion of grey matter for each participant to be calculated. As expressing this value as a proportion takes between subject variability in head size into account, it may reflect a more accurate covariate compared with grey matter volume alone.

Definition of Region of Interest

Previously published PCC foci (Talairach coordinate right 8 -50 12, left -8 -50 12) were used to identify right and left PCC seed region spheres of 5mm diameter for each individual participant. The blood oxygen level dependent time series of the voxels within these spheres was averaged to generate a reference time series.

Data Analysis

Neuropsychological Analyses. Demographic, clinical and semantic and episodic neuropsychological features were analysed with between group analyses of variance. Bonferroni correction for multiple comparisons was applied to post hoc contrasts. Analyses were undertaken in Statistical Package for Social Sciences (SPSS; version 18).

Functional Connectivity Analyses. A series of analyses in the general linear model were undertaken in SPM8 to regress the spontaneous regional correlates of right and left PCC with episodic and semantic index scores. Age, sex, education and proportion of grey matter were included in each model as covariates. Sex was included as a control for gender differences in head size. These right and left PCC, episodic and semantic regression analyses were run for each separate group.

Two further models were built to test the effect of age and the effect of disease (MMSE) across all participants. Sex, education and proportion of grey matter were included in the age model as covariates and the disease model also co-varied for age.

To correct for multiple comparisons and avoid false rejection of true positives in addition to false positives for all analyses statistical significance was set a threshold of $p < 0.05$, false discovery rate (FDR).

Anatomical regions that were significantly correlated with PCC connectivity and were identified using the Talairach Daemon Client (http://ric.uthscsa.edu/projects/tdc/) following conversion of the Montreal Neurological Institute coordinates extracted from the SPM analyses into Talairach coordinates.

Results

Participant Characteristics

The demographic, clinical variables and neuropsychological scores for each group can be found in Table 2. As discussed in the methods section, due to the significant differences in age between all groups and education between the YHC with MCI and OHC, these variables were included in the regression analyses as covariates. In line with detecting global cognitive decline and disease severity MMSE scores were only significantly different between the MCI group and both control groups.

Similarities scores were the only measure that did not distinguish persons with MCI from OHC and YHC participants. The other two semantic measures and subsequent semantic index scores were sensitive to abnormal ageing as persons with MCI had significantly different scores to OHCs and YHCs. The semantic measures did not differ between OHCs and YHCs.

Similarly Spatial Span and Visual Supraspan were only sensitive to abnormal ageing as MCI patients' scores were significantly lower than OHCs and YHCs and there were no differences between the scores of control groups. However, whilst all episodic memory scores were sensitive to abnormal ageing, as demonstrated by differences between MCI and control groups, they were not specific as Verbal Paired Associates, Logical Memory Total, Rey Delay and the episodic index revealed significant differences between the OHC and YHC.

	MCI (n =22)	**OHC (n = 21)**	**YHC (n = 20)**	**f/ x2**	**Partial eta squared**	**p**	**Post hoc comp (p<0.05*)**
Age	75.33, 6.96	66.36 7.45	38.25 8.24	133.07	0.816	0.001	MCI>OHC, OHC>YHC, MCI > YHC
Education	9.95, 4.21	10.86, 4.89	16, 3.23	12.34	0.29	0.001	YHC > OHC, YHC > MCI
Gender (M:F)	8:14	11:10	8:12	1.22		0.542	NS
MMSE	27.01, 2.26	29, 1.51	29.4, 0.75	11.79	0.28	0.001	MCI < OHC, MCI < YC
Semantic Memory							
Semantic Fluency	30.62, 8.94	44.45, 10.71	48.2, 6.72	21.97	0.42	0.001	MCI < OHC, MCI < YHC
Similarities	19.19, 4.97	20.27, 5.66	20.2, 3.09	0.34	0.01	0.711	NS
Confrontation Naming	18.67, 1.43	19.59, 0.80	19.6, 0.82	5.4	0.15	0.007	MCI < OHC, MCI < YHC
Semantic Index mean Z score	-1.88, 1.35	-0.28, 0.75	0.00, 0.68	22.40	43	0.001	MCI < OHC, MCI < YHC
Episodic Memory							
Verbal Paired	11.05, 3.51	13.93, 4.00	16.33, 3.52	10.52	0.26	0.001	MCI < OHC, MCI < YHC, OHC < YHC

	MCI (n =22)	OHC (n = 21)	YHC (n = 20)	f/ x2	Partial eta squared	p	Post hoc comp (p<0.05*)
Associates							
Logical Memory Total	15.42, 7.26	22.39, 6.38	31.8, 8.49	25.25	0.46	0.001	MCI < OHC, MCI < YHC, OHC < YHC
Rey Delay	9.29, 5.40	15.70, 5.90	21.98, 6.92	22.25	0.43	0.001	MCI < OHC, MCI < YHC, OHC < YHC
Spatial Span	4.33, 0.73	5.00, 0.76	5.50, 1.00	10.14	0.25	0.001	MCI < OHC, MCI < YHC
Visual Supraspan	15.25, 8.10	21.02, 5.57	23.20, 6.02	7.95	0.29	0.001	MCI < OHC, MCI < YHC
Episodic Index Z score	-1.54, 0.65	-0.65, 0.64	- 0.00, 0.67	29.05	0.49	0.001	MCI < OHC, MCI < YHC, OHC < YHC

*$p < 0.05$ with Bonferroni Correction

Table 2. Means, Standard Deviations, ANOVA (F) and Chi Square for demographic and neuropsychological variables for MCI, OHC and YHC groups

Relationship between semantic and episodic memory performance and PCC connectivity

At the FDR corrected threshold, voxel-wise regression analysis revealed clusters of significant positive associations between the episodic memory index and bilateral PCC connectivity in the MCI group and left PCC connectivity in the YHC group. No associations between PCC connectivity and semantic index scores were evident for the MCI or YHC groups. The correlation strength, brain regions, Brodmann areas and Talairach coordinates for these relationships can be found in Table 3, the regions of regression can be seen in Figures 2 and 3.

In detail, for the MCI group lower episodic memory was significantly positively associated with lower functional connectivity between the right PCC and right cuneus, left precuneus, left tuber, declive and bilateral culmen. Decreased connectivity of the left PCC with the right culmen and cerebellar tonsil of the cerebellum was significantly positively associated with episodic memory impairment.

For the YHC group, significant positive associations were observed between right PCC connectivity with the right cingulate and caudate and episodic memory performance.

A significantly inverse association was found for the connectivity of the right PCC with the right medial frontal, middle frontal, bilateral caudate nucleus and left insula and the with episodic memory index score. Similarly, negative correlations between lower left PCC connectivity with the bilateral insula and caudate nucleus, left lingual gyrus and left frontal sub gyral areas and higher episodic memory scores were evident for this group. The semantic index was also inversely associated with left PCC connectivity with the left claustrum, insula and caudate nucleus in the OHC sample.

		+ve/-ve Correlation	Brain area	Left/ Right	Brodmann area (BA)	Cluster size	Z values at local maximum	p values FDRcorr	r values	Talairach Coordinates x	y	z
Episodic Index Scores												
MCI	**Right PCC**	**+**	Cuneus	R	18	1058	4.42	0.001	0.87	0	-82	27
			Precuneus	L	31		4.14		0.85	-18	-74	24
			Cuneus	R	19		3.99		0.83	10	-82	-35
			Tuber	L		806	4.06	0.001	0.84	-36	-63	-24
			Culmen	L			3.94		0.83	-26	-44	-27
			Declive	L			3.60		0.79	-18	-71	-18
			Culmen	R		527	3.84	0.005	0.82	28	-50	-24
			Declive	R			3.75		0.81	28	-61	-21
			Culmen	R			3.60		0.79	22	-55	-19
	Left PCC	**+**	Culmen	R		395	4.22	0.046	0.86	26	-52	-22
			Culmen	R			3.82		0.82	32	-54	-27
			Cerebellar tonsil	R			3.56		0.78	28	-62	-31

		+ve/-ve Correlation	Brain area	Left/ Right	Brodmann area (BA)	Cluster size	Z values at local maximum	p values FDRcorr	r values	Talairach Coordinates x	y	z
YHC	**Right PCC**	+	Cingulate	R	24	471	3.86	0.005	0.83	0	5	31
			Cingulate	R	24		3.48		0.70	4	-0	37
			Caudate	R			3.40		0.77	6	24	8
OHC	**Right PCC**	-	Medial Frontal	R	9	408	5.06	0.019	0.92	18	44	15
			Caudate	R			4.91		0.91	20	21	3
			Middle Frontal	R	10		3.52		0.77	34	37	7
			Caudate	L		1565	4.04	0.001	0.83	-20	-20	27
			Insula	L	13		3.83		0.81	-28	-36	25
			Caudate	R			3.75		0.80	12	-14	27
	Left PCC	-	Insula	R	13	1995	4.05	0.001	0.84	38	-5	21
			Caudate	R			4.05		0.83	20	-10	26
			Lingual Gyrus	L	18		4.05		0.83	-4	-70	-1
			Insula	L	13	634	3.68	0.001	0.79	-24	-30	25
			Caudate	L			3.65		0.79	-18	-20	24
			Frontal Sub-									

		+ve/-ve Correlation	Brain area	Left/ Right	Brodmann area (BA)	Cluster size	Z values at local maximum	p values FDRcorr	r values	Talairach Coordinates x	y	z
			Gyral	L	6		3.46		0.77	-18	1	51
Semantic Index												
OHC	**Left PCC**	-	Insula	L	13	637	4.43	0.002	0.87	-28	-28	22
			Claustrum	L			3.38		0.76	-36	-8	-9
			Caudate	L			3.32		0.75	-24	-18	24

Table 3. Areas of significant correlation with PCC connectivity and Episodic and Semantic Index Scores
$p < 0.01$ (voxel level FDR corrected)

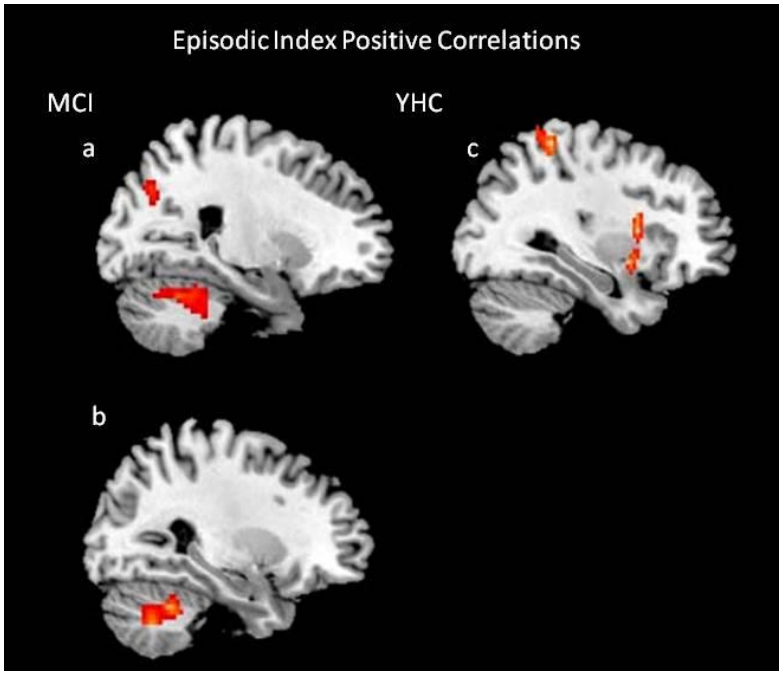

Figure 2. Regions significant positive correlation with PCC connectivity and episodic index scores. a. Right PCC correlation with precuneus and cerebellum, b. Left PCC correlation with cerebellum, c. Right PCC association with the cingulate and caudate. Images are presented in neurological convention (R/R)

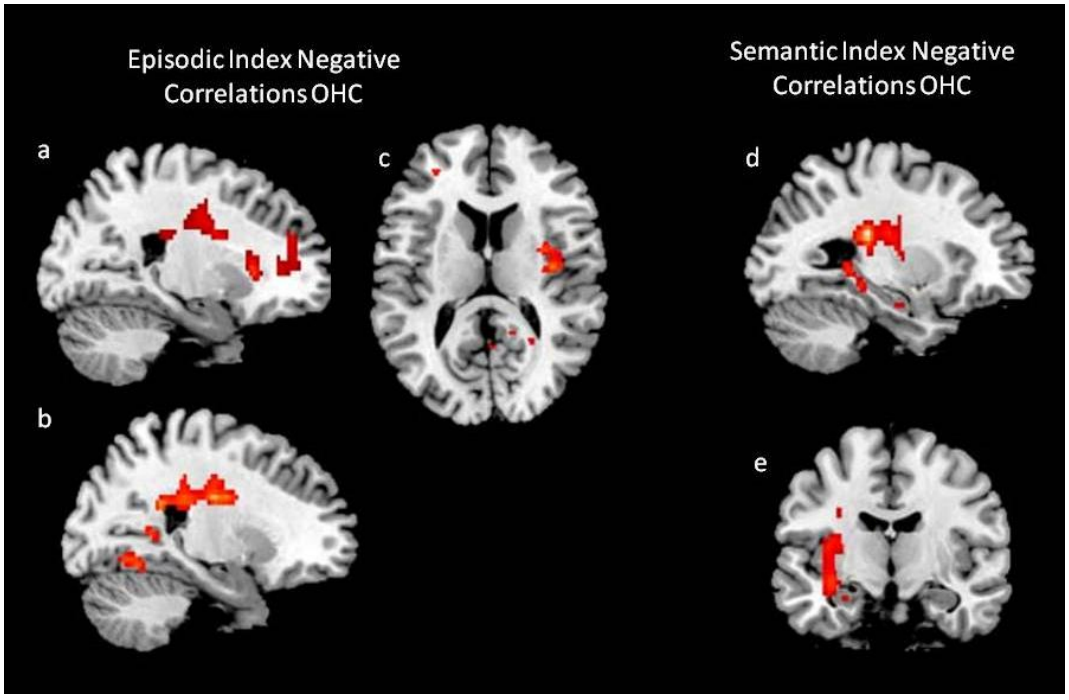

Figure 3. Regions significant negative correlation with PCC connectivity and episodic and semantic index scores. a. Right PCC correlation with medial and middle frontal and caudate, b. Left PCC correlation with caudate and lingual gyrus, c. Left PCC association with the insula d. Left PCC correlation with the caudate, e. Left PCC correlation with the claustrum. Images are presented in neurological convention (R/R)

Relationship between Age and Disease Severity with PCC Connectivity

In order to explore the way in which PCC connectivity varies as a function of ageing and disease severity, separate regressions were computed across all participants. The same FDR correction was applied to all results. The correlation strength, brain regions, Brodmann areas and Talairach coordinates for these relationships can be found in Table 4, Figure 4. Displays the brain regions of significant correlation with age and disease.

A significant positive correlation was found between increased connectivity between the left PCC and bilateral cingulate, left fusiform gyrys, right middle temporal and right superior occipital gyrus and older age.

Increased disease severity, as demonstrated by lower MMSE scores was significantly inversely associated with higher right PCC connectivity with the right precentral gyrus, claustrum and inferior frontal gyrus. Similarly, the connectivity of the left PCC with the left precentral gyrus, insula and parahippocampal grus was negatively associated with MMSE scores.

		+ve/-ve Correlation	Brain area	Left/ Right	BA	Cluster size	Z values at local maximum	p values FDRcorr	r values	Talairach Coordinates x	y	z
Age	**Left PCC**	**+**	Posterior Cingulate	L	29	1155	5.13	0.001	0.85	-8	-46	14
			Posterior Cingulate	R	29		4.27		0.79	6	-42	17
			Fusiform Gyrus	L	19		3.91		0.76	-22	-65	-9
			Middle Temporal	R	39	394	3.82	0.05	0.75	34	-59	20
			Middle Temporal	R	39		3.44		0.71	44	-71	16
			Superior Occipital	R	19		3.38		0.70	38	-73	24
MMSE	**Right PCC**	**-**	Precentral Gyrus	R	6	3142	5.17	0.001	0.85	34	5	31
			Claustrum	R			4.82		0.83	36	-2	7
			Inferior Frontal	R	47		4.64		0.82	38	33	2
	Left PCC	**-**	Precentral Gyrus	L	6	9534	5.02	0.001	0.84	-34	5	31
			Insula	L	13		4.83		0.83	-40	14	6
			Parahippocampal Gyrus	L	30		4.77		0.83	-30	-52	9

Table 5. Areas of significant correlation with PCC connectivity and years of age and MMSE scores
$p < 0.01$ (voxel level FDR corrected)

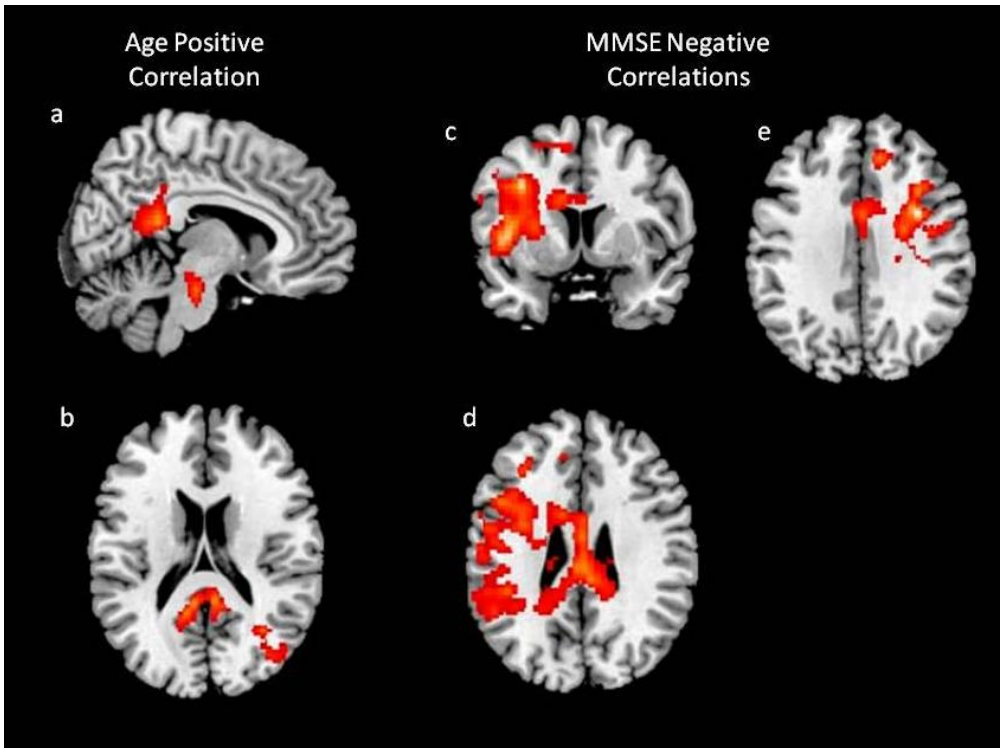

Figure 4. Regions significant positive and negative correlation with PCC connectivity age and MMSE. a. Left PCC correlation with posterior cingulate b. Left PCC correlation with bilateral cingulate and superior occipital lobe, c. Left PCC association with the right insula d. Left PCC correlation with insula and parahippocampal gyrus, e. Right PCC correlation with precentral gyrus and inferior frontal lobe. Images are presented in neurological convention (R/R)

Discussion

As predicted semantic scores were sensitive and specific to abnormal ageing as they were able to discriminate MCI from both control groups and were not susceptible to the effects of normal ageing. Visuospatial episodic memory tests were also invulnerable to effects of ageing. However, whilst the verbal measures of episodic memory were sensitive to the presence of disease as they differentiated MCI from OHC, this discrimination was nonspecific as this index also detected an ageing effect since the OHC scores were significantly lower than the YHC scores. These behavioural results are in line with the findings from neuropsychological studies of early markers of pathological alterations in prodromal AD (Gardini et al., 2013; Rodriguez et al., 2012) and support the inclusion of semantic measures in addition to measures of episodic memory in assessments to distinguish between normal and abnormal ageing (Venneri et al., 2011).

Investigation of the neuropsychological correlates of PCC connectivity revealed several interesting significant associations that may have clinical relevance. Whilst the episodic index was significantly associated with PCC connectivity with several important memory structures in all groups, semantic memory was only associated with the spontaneous correlations between the PCC and regions in the insula, claustrum and caudate nucleus in the OHC group. No significant relationships were detected between the semantic index and resting state activity of the PCC in the MCI or YHC groups.

The lack of association between PCC connectivity and semantic scores in the MCI and YHC groups may suggest that the PCC is not an appropriate seed to detect the neural substrates that support semantic memory encoding and retrieval. However, the fact that semantic memory was significantly negatively associated with PCC connectivity in the OHC group suggests that anticorrelation of the PCC may reveal a potential mechanism of healthy ageing. Studies of the DMN in normal ageing have demonstrated that there are specific differences in properties of this network between older and younger individuals (Damoiseux et al., 2008; Jones et al., 2011). As resting state networks may also be modulated by learning through alterations in plasticity (Stevens, Buckner & Schacter, 2010) these circuits may demonstrate signs of cognitive efficiency in older persons. Contrary to a deficit hypothesis of ageing, healthy older persons can demonstrate increases in cognitive efficiency compared with younger persons (Rabbit, 1993). For instance, compared with younger controls, healthy older individuals have been found to recruit fewer brain regions whilst achieving at least an equal performance as younger comparison groups while performing a semantic association task under fMRI conditions (McGeown et al., 2009a).

The structural associations with PCC connectivity and semantic memory are behaviourally and anatomically meaningful in this context. The claustrum has topographical connections with posterior and anterior hubs of the DMN and is important to multi modality sensory integration (Banati, Goerres, Tjoa, Aggleton, & Grasby, 2000) and integral for attaining conceptual congruency

(Naghavi, Eriksson, Larsson, & Nyberg, 2007). Volume change in this region has also been associated with impaired category fluency and confrontation naming in studies of AD and MCI (Gardini et al., 2013). Similarly the insula specialises in integrative functions, and is anatomically and functionally connected to paralimbic memory structures, although is not intrinsic to the DMN, it is functionally and anatomically associated with this network. The caudate nucleus is also characterised as an important integrative hub of semantic retrieval circuits and it may be especially important to the suppression of incorrect retrieval (Hart et al., 2013). The similarities across these systems in terms of associations with semantic and epsiodic memory, and their functional roles as integrative hubs and implications for mechanisms of surveilling/ monitoring of correct appropriate retreival suggests anticorrelation with these regions may be in line with an efficient memory retireval system which can bypass congruence, valence or fact checking. By contrast, the positive assocation between PCC-caudate connectivity in the younger group may suggest these individuals still need to recruit integrative areas to supress and correct memory retreival (Hart et al., 2013).

Whilst the hypothesis that semantic memory is sensitive and specific to abnormal ageing because it does not decline in normal ageing was in line with the neuropsychological results, the imaging data revealed that something more complex and interesting subserved the semantic ability of older persons who are neurologically healthy. This data suggested the functional connectivity supporting semantic memory *is* actually sensitive to normal ageing and semantic memory appears to stabilise in healthy ageing and semantic memory ability in healthy ageing has an important signature of neural efficiency. Similarly whilst episodic memory was found to be statistically but not clinically lower in normal ageing, there was also evidence of cognitive efficiency in the healthy older persons. Such neural signatures, which are not detectable in neuropsychology alone further attest to the application of combined imaging and neuropsychological approaches to the investigation of normal and abnormal ageing.

This line of reasoning is consitent with the inverse correlations detected in the OHC group with right and left PCC connectivity with the caudate nucleus and insula and episodic index scores. The inverse association between the mediation of episodic memory by the connectivity between frontal regions and right and left PCC connectivity is in line with the frontal hypotheses of ageing (Mittenberg, Seidenberg, O'Leary & DiGiulio, 1989). This would suggest that as the frontal lobes are less vulnerable to the effects of ageing and disease as individuals age normally and abnormally, the frontal lobes begin to assume more responsibility for anatomcial and cogntive losses. Evidence for this can be seen in the frequent finding that increased frontal connectivity is associated with disrupted PCC connectivity with the medial temporal lobes in persons with AD and MCI (Jones et al., 2011; Koch et al., 2012). Additionally, increased PCC connectiviy with the PCC in episodic memory encoding and retrieval may be an adaptive function of normal ageing which may protect amyloid vulnerable regions from the metabolistic consequences of overuse (Buckner et al., 2005; 2008).

Similarly evidence of frontal compensation was suggested by the inverse relationship between disease severity as measured by the MMSE and PCC connectivity with bilateral frontal regions. The known distibution of these scores between the MCI and contol groups suggests the higher MMSE scores of the YHC and OHC are associated with lower connectivity of frontal regions in episodic encoding and retrieval whereas the lower scores of the MCI group are associated with higher connecitivity between the PCC and frontal regions, suggesting a possible reliance on recruitment of additional compensatory support. Similalry, the healthy control groups are less dependent on claustrum and insula integration than the MCI group whose contribution to variance in the lower MMSE scores was correlated with higher linear dependance on these structures.

In line with the main hypothesis, lower connectivity between PCC connectivity in posterior DMN regions was associated with episodic memory impairment. For the MCI group lower episodic memory index scores indicative of episodic memory impairment were significantly positively

correlated with lower connectivity between the PCC and the cuneus and precuneus. This pattern of findings appears to be especially robust and replicable across studies and disruption of connections between these regions may reflect a reliable alteration in disease progression (He et al., 2007; Bai et al., 2008; Bai et al., 2009). The positive association between episodic memory impairment and the connectivity between the PCC and regions of the cerebellum is anatomically and behaviourally interesting. Originally the cerebellum was thought to be primarily responsible for motor function, however it is now known to mediate several linguistically based higher order cognitive functions. Several studies have demonstrated associations between the cerebellum and episodic memory (Desmond & Fiez, 1998). In cognitively healthy individuals the cerebellum was found to be associated with silent recall of episodic autobiographical memory in a functional imaging study (Andreasen et al., 1999). This task is convergent with the functions of the DMN in the spontaneous retrieval of episodic and autobiographical memories in the resting state and is in line with the association of PCC-cerebellar connectivity and episodic memory. Similarly the cerebellum has been found to mediate self referential episodic retrieval and maintenance of self concept (Fossati et al., 2004) and such findings converge with cerebellar connections within a system that subserves these referential processes, the DMN.

Across the whole group, older age was correlated with increased left PCC connectivity in the bilateral posterior cingulate, fusiform, middle temporal and superior occipital, as MMSE was controlled in the regression, these findings are statistically independent from the older age of the MCI group compared to the OHC sample. The association of higher age with higher connectivity in posterior cingulate, fusiform and middle temporal gyrus is opposite to the association with lower connectivity of these regions and memory impairment in MCI (Jin et al., 2012; Yan et al., 2013). This difference may reflect an important dissociation of connectivity in normal and abnormal ageing.

Normally the PCC and parietal lobes should deactivate when an individual needs to encode information (Duverne, et al., 2008) and activate when retrieving episodic and autobiographical recall (Svoboda, et al., 2006). Abnormal ageing is associated with a slower deactivating process or less deactivation all together. In the context of neurodegeneration, the burden of the constant switching between the encoding and retrieval demands of everyday life may become a metabolic issue (Sperling et al., 2009) which may increase amyloid production (Cirrito et al. 2005). This may explain why the PCC is so susceptible to early pathology and why this affects memory encoding and retrieval. The finding may also suggest the PCC is a better indication of early pathological processes than the medial temporal lobes and may also account for the way in which the PCC is more susceptible to amyloid deposition than medial temporal structures due to the PCC's role as a memory hub which is in a constantly switching state (Sperling et al., 2009). Originally the memory impairment in AD was thought to be due to MTL pathology, however the current findings are in line with recent work which suggests that as the PCC is a hub in the distributed network which subserves memory in the resting state, it may be a better cognitive and imaging marker of amyloid insult (Sperling et al., 2009).

Limitations

There are several limitations to the current study. Whilst the sample is of an average size in comparison with similar studies it may not have had sufficient power for all relationships to remain significant in corrected models with appropriate covariates. However, the decision to correct with FDR rather than family wise error may have limited the loss of true positives. Another potential issue is the significant age difference between the MCI and OHC group and the significant difference in education between the YHC and MCI and OHC groups. However these issues should have no bearing on the results as they were controlled in the statistical analyses.

The decision to focus on the PCC as a seed ROI may also impose limitations on the findings. Whilst the results suggest that important indices of memory are associated with connectivity between the PCC and several other brain regions especially in the DMN, the PCC ROI means that only functional relationships that involve the PCC were detected. This implies there may be other functional relationships that subserve memory in normal and abnormal ageing that may not have been detected by the current methods. However, the decision to use the PCC was based on its important role in memory encoding and retrieval, its position as an anatomically and functionally central hub of the DMN and due to its susceptibility to early pathological change. Additionally, the current method allowed the functional relationships between key memory domains and PCC connectivity with any other region of the brain to be detected. Future work, combining hypothesis based ROI analyses with whole brain Independent Component Analysis, could allow the relationship with cognition and the full scale information of the whole brain to be observed in unbiased but subsequently theoretically constrained manner.

A main issue in resting state studies is the fact that it is not possible to control for the random subconscious thoughts of participants. A possible method to minimise this in future work would be to ask participants to recall the spontaneous and random thoughts after scanning and enter these factors into regression analyses (Bai et al., 2009). However, this approach is not without limitation as participant subjective self report may not be entirely accurate. Future work is needed in order to understand the contribution of spontaneous thought and processing to functional connectivity. Similarly, between-subject differences in processes like anxiety may also impact on functional connectivity and future studies are needed to investigate these potential relationships. There may also be a level of interference from cardiac and respiratory artefacts. Whilst the sampling rate was chosen to minimise this, future studies could measure these rhythms during image acquisition and covary them out of analyses (Bai et al., 2009).

Although standardising all scores to the healthy control group will have made the data more normally distributed, a potential issue could have been introduced by the use of index scores. Whilst this approach was chosen to reduce random error, floor and ceiling effects and the issue of multiple comparisons, some test-specific brain-behaviour relationships may have been lost through combination in an index. Although the semantic and episodic tests that formed the indices are reliable and valid measures, as they measure subtly different aspects of these memory abilities in different domains the specificity that individual neuropsychological tests may have for detecting abnormal neural relationships between certain brain regions (Rodriguez-Ferrerio et al., 2012) may be lost in a more general composite score. In future work whether individual scores or indices reveal the most salient differences in brain behaviour associations should be investigated in a series of exploratory analyses.

In addition, future studies with longitudinal designs are needed in order to ascertain whether the relationships between the PCC and cognition are mechanistically associated with conversion to AD and if disrupted functional connectivity of the PCC is a meaningful biomarker of memory impairment. Follow up of this sample would also allow appraisal of sample heterogeneity to be made and subsequent analyses could investigate differences in PCC connectivity with cognition in persons with MCI who remain stable and those who convert to AD. It would also be of merit to understand how behavioural treatments such as cognitive stimulation are expressed in modulations of the neuropsychological correlates of the PCC and other resting state networks. In addition, as discussed in the literature review, PCC connectivity to important encoding and retrieval structures in the default mode may be sensitive to other important relationships that may distinguish persons with MCI from healthy controls of the same age such as self reference, autobiographical memory, awareness and theory of mind. The convergence of these complex cognitive functions with the PCC cerebellar connectivity demonstrated here suggests further investigation of these functions would be clinically meaningful.

Despite the limitations, this study has a number of strengths which attest to the reliability of the findings. As the proportion of each individual's grey matter volume was statistically controlled in all analyses, the differences demonstrated in the analyses cannot be attributed to grey matter density or atrophy. This suggests these relationships are functionally mediated and may reflect earlier, potentially treatable biomarkers. The application of a conservative statistical correction and stringent statistical correction for important covariates suggests these findings can be considered reliable. Additionally the fact that these findings survived such correction and covariance in relatively small samples suggests a level of robustness. This is further corroborated by consistency with findings in previous work in cognitively healthy individuals and in AD and MCI research.

This is also the first study to compute these regressions in a model which includes the variance of the functional connectivity with the whole brain in each sample. Typically studies have limited analyses to the regions that were found to be statistically different between patient and control groups. Additionally these studies have regressed variance in behavioural measures against the extracted Z score of the region of between group difference at the cluster level. This prohibits an understanding of within group brain behaviour relationships and may be unable to detect many important associations for understanding the full information of health and disease.

Clinical implications

The study of resting state and cognitive markers in normal and abnormal ageing may identify a multimodal and valid biomarker for early detection and classification of neuropathological processes. This information in combination with an understanding of the role of DMN in cognition may contribute to more sophisticated diagnostic protocols. Recording resting state activity is also less invasive, less time consuming and less stressful than other neuroimaging techniques and findings are not obfuscated with paradigmatic confounds.

Further understanding of the relationship between the PCC and memory in normal and abnormal ageing may also guide future behavioural and pharmacological treatment rationales, which may help to delay disease onset. Such neuroimaging endophenotypes may also be applied as potential markers for the occurrence of hidden cognitive processes and provide evidence in favour of, or against, particular cognitive neuropsychiatric models of symptoms. In addition, endophenotypic measures may help refine the distinction between symptoms that are very similar at the phenomenological level such as normal and abnormal ageing.

As preventive strategies are developed and new cognitive enhancing therapies emerge, such results may also help improve definition of which domains are expected to improve in MCI populations (Traykov et al., 2007). Functional imaging results have influenced or even motivated various physical treatment approaches. For instance, apart from pharmacological intervention, the results of the proposed thesis could provide information about the pathophysiological nodes that may become the targets for physical interventions such as transcranial magnetic stimulation and deep brain stimulation. The results may also have implications for other non invasive behavioural therapies such as neurofeedback. Through this method patients can be trained in the self-regulation of brain networks, which may help overcome dysfunctional or impaired physiological processes. This technique consists in training participants to influence the neural signal from a target area while they receive online information about the amplitude of this signal (Johnston, Boehm, Healy, Goebel, & Linden, 2010). This translational technique has shown some preliminary success in chronic pain, whereby fMRI-neurofeedback, targeting the anterior cingulate cortex, has been found to significantly improve the perception of pain (deCharms et al., 2005).

In addition to neurofeedback, very recent work suggests that other non-pharmacological strategies may also prove to be effective treatment. Such strategies may fulfil the urgent need to prevent and counteract the symptoms of AD. Behavioural techniques such as cognitive stimulation might slow down cognitive decline of AD patients at the MCI stage. The evidence thus far suggests

that even at the MCI stage there is potential for neuroplasticity and targeted non-pharmacological interventions may prevent or mitigate the negative effects of AD progression. Such interventions reside at the cutting edge of evidence based practice and will no doubt become central to the role of a clinical psychologist's assessment and treatment work. Participation in cognitively stimulating activities may also be associated with reduced risk of developing AD (Landau et al., 2012). Recently, a targeted programme of intensive cognitive stimulation elicited specific improvements in controls and persons with MCI in the neural areas that are affected by AD neuropathology at a very early stage of the disease (De Marco, Meneghello & Venneri, 2012). Similarly, another recent study found that engagement in frequent cognitive stimulation may slow or even prevent deposition of beta amyloid which has been implicated in the onset and progression of AD (Landau et al., 2012).

In line with the metabolism hypothesis of AD an important line of further investigation would be to assess whether it is possible to train people to deactivate the pathologically vulnerable over worked networks.

Neuroimaging and cognitive markers may also represent more objective outcome measures. Treatment monitoring with functional neuroimaging has been successfully applied to studies of therapy (Furmark et al., 2002; Paquette et al., 2003; Straube, Glauer, Dilger, Mentze, & Miltner, 2006). It may ultimately become possible to identify likely responders to different types of therapy by their baseline patterns of neural activation, allowing for an individualised therapy. However the method is iterative, the behavioural and imaging markers must first be understood endophenotypically in order to be used to monitor salient behavioural and physiological outcomes of therapeutic intervention.

Conclusion

The results of this thesis suggest that the strength and direction of the neuropsychological

correlates of PCC connectivity with DMN memory structures and other important integrative ‘hub’ structures reveals important biomarkers for normal and abnormal ageing.

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Appendices

Appendix 1: Modified Downs & Black, checklist for measuring study quality

Question	Scoring	Score Max 32
Reporting		
1. *Is the hypothesis/aim/objective of the study clearly described?*	Yes=1 No=0	
1a. *Is a hypothesis/aim/objective of the study clearly related to brain behaviour relationships?*	Yes=1 No=0	
2. *Are the main outcomes to be measured clearly described in the Introduction or Methods section?* If the main outcomes are first mentioned in the Results section, the question should be answered no.	Yes=1 No=0	
3. *Are the characteristics of the patients included in the study clearly described?* In cohort studies and trials, inclusion and/or exclusion criteria should be given. In case-control studies, a case-definition and the source for controls should be given.	Yes=1 No=0	
4. *Are the distributions of principal confounders in each group of subjects to be compared clearly described?* A list of principal confounders is provided.	Yes=2 Partially=1 No=0	
5. *Are the main findings of the study clearly described?* Simple outcome data (including denominators and numerators) should be reported for all major findings so that the reader can check the major analyses and conclusions. (This question does not cover statistical tests which are considered below).	Yes=1 No=0	
6. *Does the study provide estimates of the random variability in the data for the main outcomes?* In non-normally distributed data the inter-quartile range of results should be reported. In normally distributed data the standard error, standard deviation or confidence intervals should be reported. If the distribution of the data is not described, it must be assumed that the estimates used were appropriate and the question should be answered yes.	Yes=1 No=0	
7. *Have actual probability values been reported(e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?*	Yes=1 No=0	
External validity. All the following criteria attempt to address the representativeness of the findings of the study and whether they may be generalised to the population from which the study subjects were derived.		
8. *Were the subjects asked to participate in the study representative of the entire population from which they were recruited?* The study must identify the source population for patients and describe how the patients were selected. Patients would be representative if they comprised the entire source population, an unselected sample of consecutive patients, or a random sample. Random sampling is only feasible where a list of all members of the relevant population exists. Where a study does not report the proportion of the source population from which the patients are derived, the question should be answered as unable to determine.	Yes=1 No=0 Unable to determine=0	
9. *Were those subjects who were prepared to participate representative of the entire population from which they were recruited?* The proportion of those asked who agreed should be stated. Validation that the sample was representative would include demonstrating that the distribution of the main confounding factors was the same in the study sample and the source population.	Yes=1 No=0 Unable to determine=0	
Internal validity - bias		
10. *If any of the results of the study were based on "data dredging", was this made clear?* Any analyses that had not been planned at the outset of the study should be clearly indicated. If no retrospective unplanned subgroup analyses were reported, then answer yes.	Yes=1 No=0 Unable to determine=0	

11. *Were the statistical tests used to assess the main outcomes appropriate?* The statistical techniques used must be appropriate to the data. For example nonparametric methods should be used for small sample sizes. Where little statistical analysis has been undertaken but where there is no evidence of bias, the question should be answered yes. If the distribution of the data (normal or not) is not described it must be assumed that the estimates used were appropriate and the question should be answered yes.	Yes=1 No=0 Unable to determine=0	
12*. *Were the main neuropsychological measures used accurate (valid and reliable)?* For studies where the measures are clearly described, the question should be answered yes. For studies which refer to other work or that demonstrates the outcome measures are accurate, the question should be answered as yes.	Yes=1 No=0 Unable to determine=0	
13*. *Was the range of neuropsychological measures appropriate for hypotheses?*	Episodic Retrieval = 1 Encoding =1 Recognition =1 Learning =1 Semantic =1 Executive =1 Visuospatial = 1 Language =1 Working Memory = 1 Max =9 No =0	
Internal validity - confounding (selection bias)		
14*. *Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?* This question should be answered no if the effect of the main confounders was not investigated or confounding was demonstrated but no adjustment was made in the final analyses the question should be answered as no.	Demographic confounds =1 Imaging Confounds = 1 Grey matter control whole brain = 2 Grey matter control ROI =1 Brain behaviour confounds =1 Max = 6 No =0 Unable to determine =0	
Power		
15*. *As there are no studies with power analysis, did the sample appear to have sufficient power to detect a clinically important effect?*	Size of *smallest* MCI group A n5 – n10 = 0 B n11–n15 = 1 C n16–n20 = 2 D n21–n25 = 3 E n26–n30 = 4 F n31+ = 5	

Appendix 2: Context of secondary data and student's personal role in identification of the research questions and analyses etc.

As retrospective data was used in this thesis it is necessary to clarify the author's personal and original input to the design of the study. The original study from which the MCI patient data was obtained was a within subjects design which only included the MCI patient group. This study only used the neuroimaging data and the patients' disease severity scores. The reason why these patients have a full battery of neuropsychological data is because this is routinely collected clinically for assessment and diagnostic purposes. The site in which this data was collected is a specialist clinical and research facility and the patients provided consent for their clinical data to be used in future research, they also provided consent for their experimental fMRI data to be used for studies subsequent to the original experiments.

It would be feasible to just use this patient group to look at the association between the DMN and neuropsychological cognitive impairment in abnormal ageing. However, in order to see how this is modulated by MCI, I decided that a control group of healthy age matched individuals was necessary. In addition, in order to understand how this is modulated by age, I also decided to select a younger control group. I selected the age matched and young control participants from a large volunteer database of neuropsychology and neuroimaging data which has been collated from many researchers over many studies and from participant drives. These participants are asked to undertake each neuropsychological test that comprises part of the patient diagnostic battery. The original study was a within subjects design which only included the MCI patient group. The context of the current thesis is the first time that these data have been put together in a study.

In addition to choosing to undertake a cross sectional approach to the study design, I selected specific tests based on my hypotheses from a large battery of neuropsychological data that had been collected for the patients and participants. I could have chosen any number of neuropsychological domains to correlate with DMN connectivity, such as attention, executive function, working memory or visuospatial ability however, based on my review of the literature, I selected tests which measure episodic and semantic memory. Rather than looking at the relationship between the DMN and specific tests, I decided to create separate index scores devised from relevant components of certain tests in the battery, for episodic memory, semantic ability and executive function. In summary, whilst I was not involved in data collection, I was the first person to bring these participants together in this manner, I selected age matched and younger controls, I chose the cross sectional design so I could look at ageing as well as disease status and I made hypothesis driven and clinically relevant neuropsychological index scores. In addition, I chose the neuroimaging methodology, opting to take a region of interest hypothesis driven approach as opposed to a whole brain study. Finally I used the literature to select the most salient region

of interest based on my hypotheses of neuropsychological performance in health and disease – the posterior cingulate cortex.

Appendix 3: Ethical Approval

FONDAZIONE OSPEDALE SAN CAMILLO - Iscritta Prefettura di Venezia: Reg. P.G. n. 409
OSPEDALE NEURORIABILITATIVO
ISTITUTO di RICOVERO e CURA a CARATTERE SCIENTIFICO
Sede Legale: 30126 VENEZIA-LIDO, Via Alberoni, 70
Tel. 041.2207111 Fax 041.731330 C.F. 94071440278 P.I. 03953700279
Comitato Etico per la Sperimentazione

Venezia 28 giugno 2011
Rif. CE: Protocollo 11.07

Preg.ma
Prof.ssa Annalena Venneri
IRCCS San Camillo

Oggetto: Progetto 11.07 Stimolazione cognitiva nel declino cognitivo
Parere del Comitato Etico

Preg.ma Prof.ssa Venneri,

ho il piacere di comunicarLe che il Comitato Etico per la Sperimentazione dell'IRCCS San Camillo, nella seduta del 16 giugno u.s. ha espresso PARERE FAVOREVOLE all'effettuazione dello studio a condizione che entro la prossima seduta venga consegnato un protocollo formulato secondo le sue caratteristiche specifiche e non come scheda di progetto in particolare ponendo attenzione a giustificare la numerosità campionaria, inserendo una randomizzazione dei pazienti ai gruppi sperimentali e distinguendo chiaramente i moduli del consenso informato. Si ricorda inoltre di osservare quanto previsto dalla normativa vigente e regionale nonché dai regolamenti aziendali.
Allego alla presente estratto del Verbale della seduta.

A disposizione per eventuali chiarimenti, La saluto cordialmente.

Il Presidente
Don Corrado Cannizzaro

Appendix 4: Ethical Approval University of Sheffield Email

Ethics and Governance for a retrospective study Inbox x

PD Bruen <pcp10pdb@sheffield.ac.uk> 11/10/2012
to P.Sheeran

Dear Paschal

Thank you for your advice regarding whether I need to apply for university ethics for my DClinPsy dissertation.

I had wondered if I needed any further ethical approval from this university as I am analysing anonymous retrospective data from a study that was conducted at San Camillo Hospital in Italy. Ethical approval for further analyses was part of the original approval. .

You suggested that I put this in writing so I could include written confirmation of your decision that this study does not need additional ethical approval in my site file.

I also wondered if you could advise about a governance issue, I had thought San Camillo would be the governance sponsor but my supervisor told me that this concept does not exist in Italy, she also wondered if a governance sponsor was relevant for retrospective data.

Many thanks

Kind regards

Peita

p.sheeran@sheffield.ac.uk <paschal.sheeran@googlemail.com> 11/10/2012
to PD

Dear Peita,

I see no need for Department Ethics Approval on top of the ethics approval that has already been obtained for the study.

Please consult Andrew Thompson regarding the governance matter; this is outside my remit.

Regards,

Prof Paschal Sheeran
Chair, DESC

Appendix 5: Scientific Approval Letter

The University Of Sheffield.

Department Of Psychology.
Clinical Psychology Unit.

Doctor of Clinical Psychology (DClin Psy) Programme
Clinical supervision training and NHS research training & consultancy.

Clinical Psychology Unit
Department of Psychology
University of Sheffield
Western Bank
Sheffield S10 2TP UK

Telephone: 0114 2226570
Fax: 0114 2226610
Email: dclinpsy@sheffield.ac.uk
Please address any correspondence to Ms. Christie Harrison, Research Support Officer

Research Governance Office

16th August 2012

Dear Sir/Madam,

RE: **Project title:** Differentiating normal & abnormal ageing using a combined functional connectivity and neuropsychological biomarker

Applicant: Peita Bruen (DClin Psy trainee)
Supervisors: Prof Annalena Venneri; Dr Claire Isaac

1. Confirmation of NHS employment status
2. Confirmation of independent scientific approval
3. Confirmation of indemnity of enclosed Research Project

I write to confirm that the enclosed proposal forms part of the educational requirements for the Doctoral Clinical Psychology Qualification (DClin Psy) run by the Clinical Psychology Unit, University of Sheffield and that the applicant is under pressure to complete this within a designated time period.

I also confirm that the applicant, Peita Bruen, is an NHS employee and is also supervised by a clinical academic. As such the applicant has an NHS contract and has had a CRB check.

Three independent reviewers appointed by the Clinical Psychology Unit Research Sub-committee have scientifically reviewed it, including an external academic and statistician.

I can confirm that all necessary amendments have been made to the satisfaction of the reviewers, who are now happy that the proposed study is of sound scientific quality. Consequently, the University will also indemnify it, and would be happy to act as research sponsor once ethical approval has been gained.

Given the above, and in line with current NHS guidance I would ask that you exempt this proposal from further NHS scientific review and the applicant from completing an unnecessary honorary contract. The Unit already has an agreement with several local NHS Trusts (SHSRC, STH & SCH) to this effect. If you require any further information, please do not hesitate to contact me.

Yours faithfully

Dr. Andrew Thompson
Director of Research Training
Reader in Clinical Psychology & Chartered Clinical/Health Psychologist

Cc. Peita Bruen; Prof Annalena Venneri; Dr Claire Isaac

Appendix 6: Governance Sponsor Letter

The University Of Sheffield.

Department Of Psychology.
Clinical Psychology Unit.

Doctor of Clinical Psychology (DClin Psy) Programme
Clinical supervision training and NHS research training & consultancy.

Clinical Psychology Unit
Department of Psychology
University of Sheffield
Western Bank
Sheffield S10 2TN UK

Peita Breun
Trainee Clinical Psychologist
Department of Psychology
Western Bank

Clinical Psychology Unit
Department of Psychology
Western Bank
Sheffield

Telephone: 0114 22 26650
Fax: 0114 22 26610
Email: c.harrison@sheffield.ac.uk

11/10/2012

Project title: **Differentiating normal & abnormal ageing using a combined functional connectivity and neuropsychological biomarker**

6 digit URMS number: 135241

Dear Peita Breun,

LETTER TO CONFIRM THAT THE UNIVERSITY OF SHEFFIELD IS THE PROJECT'S RESEARCH GOVERNANCE SPONSOR

The University has reviewed the following documents:

1. A University approved URMS costing record;
2. Confirmation of independent scientific approval;
3. Confirmation of independent ethics approval.

All the above documents are in place. Therefore, the University now **confirms** that it is the project's research governance sponsor and, as research governance sponsor, **authorises** the project to commence any non-NHS research activities. Please note that NHS R&D approval will be required before the commencement of any activities which do involve the NHS.

You are expected to deliver the research project in accordance with the University's policies and procedures, which includes the University's Good Research Practice Standards:. http://www.sheffield.ac.uk/polopoly_fs/1.92046!/file/research_standards.pdf and Ethics Policy: http://www.sheffield.ac.uk/ris/other/gov-ethics/ethicspolicy. If the project has received NHS ethics approval then you are also expected to publish a lay summary of the project on the website of the National Research Ethics Service (NRES), as it appears in the research ethics application.

Your Supervisor, with your support and input, is responsible for monitoring the project on an ongoing basis. Your Head of Department is responsible for independently monitoring the project as appropriate. The project may be audited during or after its lifetime by the University. The monitoring responsibilities are listed in Annex 1.
Yours sincerely

Dr Andrew Thompson
Director of Research Training, Clinical Psychology Unit

cc. SUPERVISOR:
Head of Department/School: Prof Paul Overton

Appendix 7: Consent Form

Provincia Lombardo-Veneta
dell'Ordine Religioso
dei Chierici Regolari Ministri degli Infermi (Camilliani)

OSPEDALE S. CAMILLO VENEZIA – IRCCS
Istituto di Ricovero e Cura a Carattere Scientifico

Consenso informato per i trattamento dei dati personali e sensibili e per l'utilizzo di materiali biologici a scopo di ricerca
Espressione di consenso ex art. 13 del D.lgs. 196/03

Io sottoscritto (cognome e nome) .....
nato il ..... A..... .....

in qualità di:
☐ Diretto interessato

☐ Genitore, Amministratore di sostegno, Curatore/Tutore, Familiare, Prossimo congiunto, Convivente del sig./sig.ra.....

assistito dal personale medico e non medico dell'IRCCS Ospedale San Camillo di Venezia-Lido, da me scelto liberamente, e dallo stesso informato/a sui diritti e sui limiti del Decreto legislativo 196/03, previa lettura e comprensione del "foglio informativo" fornito dalla struttura,

1. **AUTORIZZO ☐** **Non AUTORIZZO ☐**

   ☐ che i **miei** dati personali e sensibili e il materiale biologico sotto precisato

   ☐ che i dati personali e sensibili e il materiale biologico sottoprecisato del/della **Sig /Sig.ra**.....(nei cui confronti mi trovo in una delle condizioni di cui sopra)
   SANGUE.....SIERO.....LIQUOR.....DNA.....MUSCOLO
   CUTE.....TESSUTO CEREBRALE.....NERVO PERIFERICO.....MRI....ESAME NEUROPSICOLOGICO

   vengano raccolti, trattati e conservati, esclusivamente ai fini di prevenzione, diagnosi e cura e prestazioni connesse per l'effettuazione dei trattamenti inerenti la diagnosi e la cura.

2. **AUTORIZZO ☐** **Non AUTORIZZO ☐**

   -che il suddetto materiale biologico venga conservato ed utilizzato anche:
   per ulteriori accertamenti diagnostici
   a scopo di ricerca scientifica

3. **AUTORIZZO ☐** **Non AUTORIZZO ☐**

   il conferimento dei dati a Mutue, Assicurazioni e Banche alle quali è stata richiesta compartecipazione economica

4. **AUTORIZZO ☐** **Non AUTORIZZO ☐**

   il conferimento dei miei dati a:
   - Medico curante
   - Medico specialista
   - Altri (specificare con nome e cognome)

5. **AUTORIZZO ☐** **Non AUTORIZZO ☐**

   la comunicazione del nominativo o il numero della stanza di ricovero, da parte della portineria o del personale dell'UO di degenza

Firma del Medico ..... Firma del paziente .....

Data.....